PRECLINICAL SAFETY EVALUATION OF TAMBIRA PARPAM

The dissertation Submitted by

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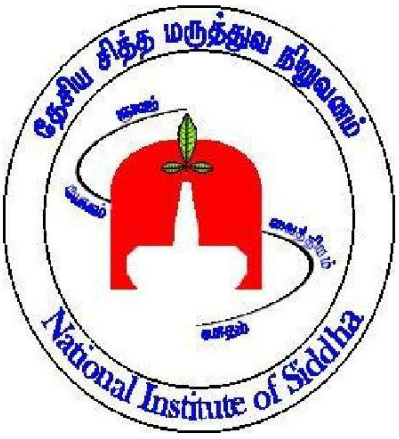

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Preclinical safety evaluation of TAMBIRA PARPAM"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.P.Shanmugapriya, M.D(S).,** Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Chennai -47, and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or another similar title.

Date: **Signature of the Candidate**

Place: Chennai-47 **Dr. K . Anbarasan**

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CONTENTS

1. INTRODUCTION

"In the great of the Vedas, there is no touch of sectarianism. It is all ages, climes, and nationalities and is the royal road for the attainment of the Great Knowledge".

Thoreau, American Thinker.

Siddha medicine is the oldest and the foremost medical systems in the world.

"சொல்லிடவே தேவிக்குச் சதாசிவன்றான்
சொல்லவே தேவியும் நந்திக்குச் சொல்ல
நல்லிடவே நந்திதன் வந்திரிக்குச் சொல்ல
நயமுடன் தன்வந்திரி யசுவினிக்குச் சொல்ல... "[2]

_ யூகி வைத்திய சிந்தாமணி 800

According to Sage Yugi, the God Shiva transmitted the knowledge of medicine to Parvati, who in turn passed it on to Nandi, from whom it was given to the first practitioners of Siddha medicine the Siddhars.

The word Siddha comes from the world Siddhi, which means an object to attain perfection or heavenly bliss. Siddhars were the ones who had attained 'Siddhi'

Siddha generally refers to Aathma Siddha that is the eight supernatural powers. Those who attained or achieved the above- said powers are known as Siddhars. There were 18 important Siddhars in olden days and they developed this system of medicine. Hence it is called Siddha Medicine.

The Siddha system of Medicine is Prevalent in South India, Sri Lanka, Malaysia, and Singapore, where the existence of Dravidian civilization was documented.This system owes its origin to the Dravidian culture which is of the Pre-historic Period. [3]

சித்தரென்பவர் ஆன்ம வித்தையில் சித்திபெற்றவர். அகத்தியரைத் தலைவராகக் கொண்ட பதினெண்மர். தமிழ் கூறும் தொல்லுலகில் தோன்றியவர்கள். பிரம்மம் முதல் கீட மீறான எல்லாச் சேதனா சேதனப் பொருள்களின் தோற்றம்,நிலைப்பேறு, மறைப்பு முதலியவைகளையும் ஒவ்வொன்றின் இயல்பு வினை, ஆற்றல் முதலிய பெருமைகளையும் பற்றிய நுணுக்கங்களை நன்கு அறிந்தவர். பிணிகள் யாவற்றையும் வெகு எளிதில் வேருடன் நீக்கும்

உபாயங்களையும் பற்பல காய கற்ப மென்னும் ஒப்பற்ற அரிய வித்தையின் இரகசியங்களையும் திறம்பட அறிந்தும் தம் சீடர்களுக்குப் போதித்தவர்கள் சித்தர்கள்.[4]

மேலும் பாகத்தினாலும், யோகத்தினாலும் திரவியங்களிலுண்டாகும் நுண்ணிய மாற்றங்களையும் தெள்ளெனக்கண்ட திவ்விய நேத்திரம் படைத்தவர்கள்.

"தான் விளைக்குங் கருஞ்சேற்றைக்
கழுவி நீக்குந் தண்புனல்போல்
இருவினைக்குந் தனுவாய் நின்ற
ஊன் விளைக்கும் ஒழிக்கும் எனும்
முறையா லிந்த உடலிருந்தன்றோ
உறுதி பயக்கவேண்டும்" [4]

சித்தர்கள் அழியுந்தன்மை வாய்ந்த மூலிகை மற்றுமுள்ள உயிரினங்களிடமிருந்து கிடைக்கக் கூடிய சரக்குகளைக் கொண்டு உறுதியையும், அழியாத தன்மையையும்,நிலைத்திருக்கும் தன்மையையும் தரவல்லதான மருந்துகளாய்ச் செய்து அவற்றை உண்டு உடலை நெடுநாள் இருக்கச் செய்து பேரின்பம் அடைந்தனர்.

The main aim of Siddhars was to certify that the container of the soul for the attainment of happiness and to reach the God. They found diseases as one of the obstacle to reach God. So they bestowed to the world the Siddha medicine to treat diseases.

Siddhars are precise in the treatment of poisons. They identified toxic substances and their antidote which is documented in Siddha literature. The astonishing knowledge of Siddhars in herbal, metal, mineral and animal products. Before going to any Siddha medical Preparation, the raw drugs. Which are purified (detoxification) and then the purified drugs are including to the medicine. The Signs, symptoms of poison in humans, the way of diagnosis of poison and treatment are explained in Nanju Maruthuvam (Siddha Toxicology). It contains general antidote for poison and treatment are explained in also the specific antidote for particular poison which is beneficial to the society.

Siddha system of medicine practiced in India has a number of remedies for various diseases as mentioned in Siddha pharmacopoeia which includes drugs of herbal mineral metal and animal origin.[5]

In our Siddha system, they are two types of medicine which include 32 internal and 32 external medicines. Among 32 internal medicines, Parpam is one among them. From the ancient period Siddha system of traditional medicine herbo mineral preparation of parpam used for the treatment of various chronic ailments.Parpams are the powder substances obtained by calcification of purified metals, minerals and animal products by the specific process.They are calcined in closed crucibles in pits and with cow dung cakes (Pudam). Generally, this method of preparation of Siddha medicines involves conversation of minerals or metals into oxide or sulfide form by various herbal treatments followed by repeated high- temperature calcination and grinding cycles. [6] The parpam thus obtained constitute ultra-small particles and are taken along with vehicles such as milk, honey, butter, ghee etc according to diseases. This makes these drugs easily assailable eliminating their harmful effects and enhancing their biocompatibility. However very few studies have been carried out to understand the phytochemical nature of these type of traditional medicines for the metal and mineral based preparations it become improve that this drug should be characterized with the help of modern instrumental techniques likely scanning electron microscopy (SEM), X-ray diffraction (XRD), Thermogravimetric analysis (TGA)' etc, based on this specification of metal- based drug can be well standardised on a scientific basis.

Traditional medicines have been the important feature in human being since the earliest times and it has recently acquired increasing importance due to its harmless nature and success.

However, these successes of our system are readily questioned by the modern science. The fact that the Siddha physicians use plants and chemicals such as Copper, Copper sulfate, mercury, arsenic, etc. which are toxic in raw form depending upon the dose, add weight age to this argument. This untrue belief of toxicity posing a major obstacle to the renaissance of Siddha medicine.

Here, Toxicology plays its role. It is the study of the untoward effects of chemicals or physical agents on biological systems. The multidisciplinary nature of toxicology is its great strength. This can be majorly subdivided into Environmental, Economic, and Medical.

Testing methods in toxicology most often involve the use of animals based on the hypothesis that results of toxicity studies in suitable animal models may be

extrapolated to humans. Over 30 countries have agreed to accept toxicity studies undertaken using OECD guidelines.[7] [8]

The Recent interest in Traditional Medicine has taken up great dimensions in changing the health care scenario across the globe.The prevalence rate of varicose vein in India estimates 15 to 20% of a population is suffering vein diseases.Breast cancer has ranked number one cancer among Indian females with the age- adjusted rate as high as 25.8 per 100,000 women and mortality 12.7 per 100,000 women. The age- adjusted incidence rate of Carcinoma of the Breast was found as high as 41 per 100,000 women for Delhi, followed by Chennai (37.9), Bangalore (34.4) and Thiruvananthapuram District (33.7). The Breast Cancer year of 1975-1977 in 75%, the year of 1984-1986 in 79%, and the year of 1996-2004 in 89% of survival rates in Breast cancer.

Tambaram is also known as 'Gunmakaalan' [9] as it is an important prescribed medicine to treat gunmam in traditional Siddha system. In the present era of globalization for the development of a world market for traditional medicines research and development is Quality control of Siddha drugs are generates a lot of problems very essential. The present study investigate the physiochemical properties of traditional Indian Siddha preparation Tambira parpam which are mentioned in the Siddha text Theran Yamaha Venba[10] widely used for treating vaginal cancer, breast cancer, varicose vein, skin diseases such as psoriasis, eczema, alopecia, vitiligo, and leprosy. Then diabetic ulcer, warts, Parkinson's. Hence the author decided to establish the safety of this formulation, which is needed in the current scientific world.

மருத்துவனின் லட்சணம்

பாரேதான் மருத்துவனும் யோகவானாய்
பாருலகில் தரும சிந்தனை யுடையோனாய்
நேரேதான் யோசனைகள் உள்ளவனாய்
நேர்மையுடன் பல பேரைக் கேட்க வல்லான்
சீரேதான் குருவினையும் நினைக்க வல்லான்
சிறப்பான சீஷனுக்கு உகந்த வல்லான்
கூறேதான் சாத்திரத்தில் வல்லனாக
குவலயத்தில் கீர்த்தியுள்ளோன் வயித்தியவானே.[11]

-அகத்தியர் 12000 காண்டம்

2. AIM AND OBJECTIVES

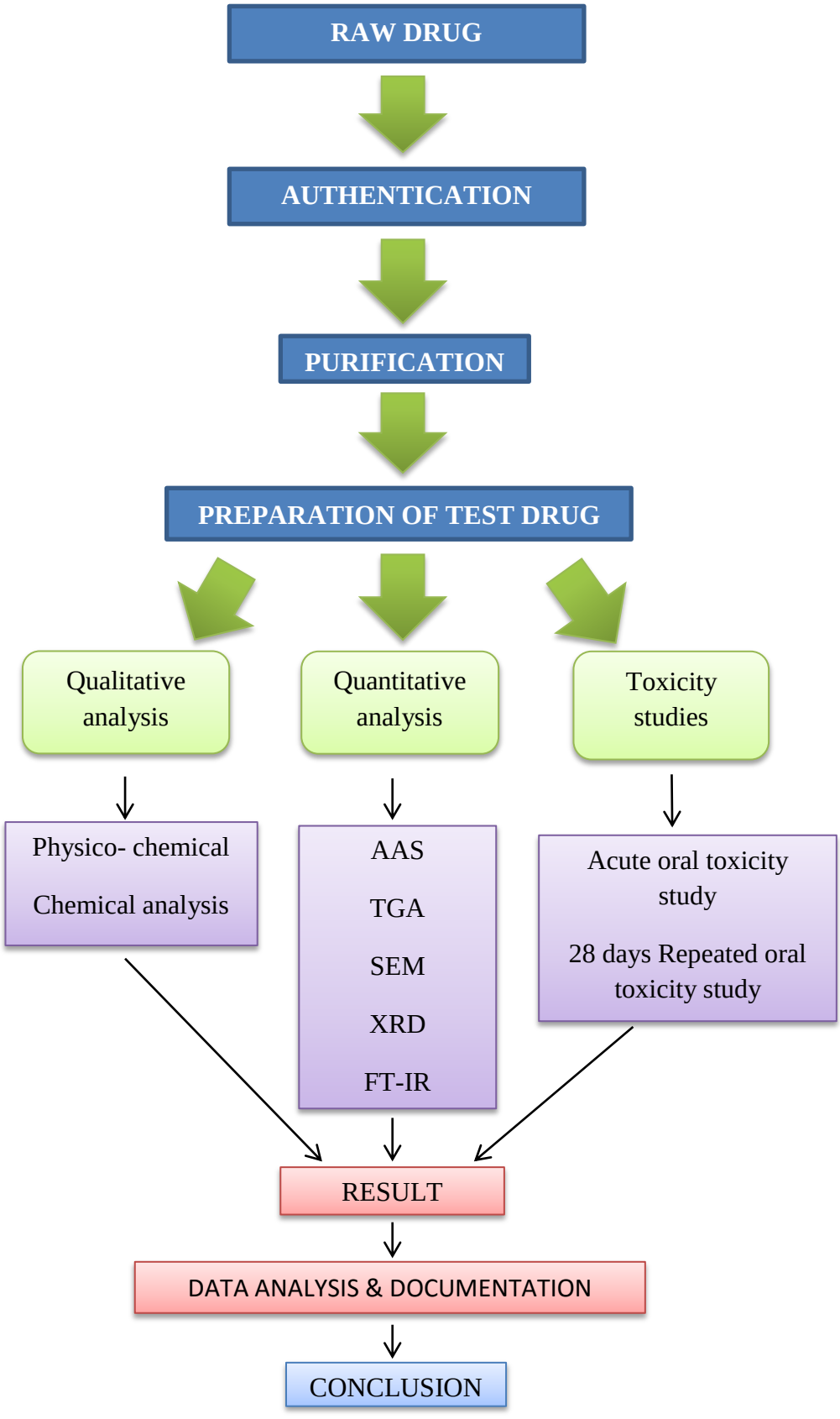
AIM

To evaluate the safety profile (Acute and 28days Repeated dose oral toxicity study) of **TAMBIRA PARPAM** in Wistar albino rats.

OBJECTIVES

- To analyze the physico-chemical properties of Tambira parpam.
- To evaluate Chemical and Spectroscopic analysis of Tambira parpam.
- To find out the safe dose of the Tambira parpam through toxicity studies on Rodents as per OECD guidelines 423 & 407.

WORK PLAN


RAW DRUG
AUTHENTICATION
PURIFICATION
PREPARATION OF TEST DRUG
Qualitative analysis
Quantitative analysis
Toxicity studies
Physico- chemical
Chemical analysis
AAS
TGA
SEM
XRD
FT-IR
Acute oral toxicity study
28 days Repeated oral toxicity study
RESULT
DATA ANALYSIS & DOCUMENTATION
CONCLUSION

3. SIDDHA ASPECT

தாம்பரம்

உலோகம் வகைகள் பதினொன்றினுள் தாம்பரமும் ஒன்று தாம்பரம் இயற்கை உலோக வகையுனுள் சேர்ந்தது.

உற்றுப்பார் தங்கம்வெள்ளி செம்பு நாகம்
உருக்கிரும்பு வெண்கலம் பித்தளை தராவும்
நத்திப்பார் காரீயம் வெள்ளீ யந்தான்
நலமாகப் பதினொன்றாய்ப் பிரிந்த தையா
-போகர் காரசாரத்துறை[9]

செம்பு செங்கபில நிறத்தையும்,மினுமினுப்புள்ள பிரகாசத்தையும், துப்புப் பிடிக்கும் தன்மையையும், சுத்தியால் அடிக்க அடிக்க அடிபட்ட இடத்தில் பட்டுப்போலத் தோன்றும் குணத்தையும் உடையது, மாசற்ற செம்பு கடினமாயிருப்பினும் கத்தி வெட்டுக்கு எளிதாய் வெட்டுப்படும். வெட்டு வாயில் செந்நிறமும் பிரகாசமும் பளிங்கு போன்ற தெளிவுங் காணப்படும். செம்பு உருகும்போது பச்சைப் புகை வெளிக்கிளம்பும்.ஆறியபின் மேற்பாகம் கருநிறமாகும். ஈரக் காற்றில்லா விடத்தில் இதன் நிறம் வேற்றுமை அடைவதில்லை. இது வெடியுப்பு திராவகம் ஒன்றிலேதான் கரையும். நெருப்பின் துணையின்றி, கறியுப்புத் திராவகத்திலேனும் கந்தக திராவகத்திலேனும் கரைவதில்லை. இதில் நாதமுண்டு இக்காரணத்தினாலேயே ஓசை எழுப்பக் கூட்டுகிற கூட்டு உலோகங்களில் இதற்கு முதன்மை இடம் கிடைக்கிறது. உஷ்ணத்தையும் கிரகிக்கும் தன்மை உண்டு.

-(குணபாடம் தாது சீவ வகுப்பு)[9]

வேறு பெயர்கள்:

- அவுதும்பரம்
- உதும்பரம்
- இரவி
- இராசி
- இரவிப் பிரியம்
- எருவை
- சீருணம்
- சீருணி
- சுற்பம்
- சுல்பம்
- தாமிரம்
- தாம்பரம்
- பரிதி
- வடு

- விடம்

மேலும் செம்பின் பெயர்கள் கீழ் கண்ட நூலில்

"செம்பின் பேரினைத்தானுஞ் செப்பக் கேளு
சிறந்த செந்தலை ராகியென்பார்
கம்பியென்ற தாம்பிர மிலேச்ச முகஞ் சுலபங்
கனமான ரத்ததாதுகமு மாகும்
பம்பியென்ற பழுப்பினமாங் காளிக்கம் பருதி
பஞ்சனா கமென்றுந் திறியம்பக மாகுஞ்
தும்பியாந் தூம்புறமும் அதுவும் பரமாகுஞ்
சொல்லியதோர் பேரெல்லாம் செம்புமாமே"[12]

-போகர் நிகண்டு 1200

- செந்தலை
- ராகி
- தாம்பிரம்
- மிலேச்சம்
- கஞ்சுலபம்
- ரத்ததாது
- கமு
- பழுப்பினம்
- காளிக்கம்
- பருதி
- பஞ்சனாகம்
- திறியம்பகம்
- தூம்புறமும்
- அதுவும்பரம்

என போகர் நூல் கூறுகிறது.

பகைச் சரக்குகள்:[13]

- காரீயம்
- வெடியுப்பு

நட்பு சரக்குகள்: [13]

- அயம்

- ❖ சூதம்
- ❖ தங்கம்
- ❖ நாகம்

நாத விந்து சரக்கு: [13]

- ❖ செம்பு-சிலாசத்து

  - **{வாதவைத்தியத்திற்கு ஆதி}**

ஆதி சரக்கு: [13]

- ❖ செம்புக்காதி-கெந்தி

<u>குணம்:</u>

- சுவை - கார்ப்பு கைப்பு
- வீரியம் - உஷ்ணம்
- விபாகம் - கார்ப்பு
- பஞ்சபூத அம்சம் - தேயு
- செய்கை - வறட்சியுண்டாக்கி, வெப்பமுண்டாக்கி, இசிவகற்றி, உடல்தேற்றி

செம்பு சத்துள்ள மூலிகைகள்:

"கொவ்வை சிவப்புக் கொடியின் சிறுகீரை
செவ்வலிகை யான்மிரி செந்தரா அய் ஒவ்வுமிடம்
மூக்கி புளிக ரணை மூதோ ரிலைக்கஞ்சந்
தேக்கவுரி மூலமிவை செம்பு."

-தேரையர் அந்தாதி[9]

கோவை, செங்கொடிவேலி, சிறுகீரை, செவ்வல்லி, கையான், செந்தரா, புளியரணை, கஞ்சா, தேக்கு, அவுரி போன்ற மூலிகைகளில் செம்பு சத்து நிறைந்துள்ளது.

செம்பு சத்துள்ள சீவ பொருட்கள்:

- இந்திர கோபப் பூச்சியிலிருந்து செம்பு சத்து எடுக்கும் முறை

  உரோமரிஷி வைத்தியம் 500[14] எனும் நூலில் விளக்கப்பட்டுள்ளது

- பூநாகம்
- மயில் இறகு
- தலை மயிர்

செம்பு சத்துள்ள உலோகங்கள்:

- அயம் - அயசெம்பு

- தங்கம் - தங்கச்செம்பு
- காந்தம் - காந்தச்செம்பு

செம்பு சத்துள்ள பாடணங்கள்:

- கந்தகம் - கந்தகசெம்பு
- தாளகம் - தாளகச்செம்பு

செம்பு சத்துள்ள உபரசம்:

- துருசு - துருசுசெம்பு[15]

செம்பின் தோஷம்:

வாந்தி, பிராந்தி, விரேசனம், இளை த்தல், தாபம், வீரியநாசம், நமைச்சல், சூலை எங்கிற எட்டுவித தோஷங்கள் செம்புக்கு உடைத்தானது.

- நல்லெண்ணெய், மோர், கோமூத்திரம் - வாந்தி
- புளித்தகாடி, கொள்ளு - பிராந்தி
- கள்ளிப்பால், பசும்பால் - இளைப்பு
- புளியின் இரசம், பழச்சாறு - தாபம்
- காட்டு வாழை, கற்றாழை - சூலை
- பசும்பால், நெய் - நமைச்சல்
- தேன் - தோஷம்
- தயிரின் ஏடு, காட்டுக்கருணை கிழங்கு இரசம் - பேதி

சுத்திமுறைகள்:

1.செம்புப் பொடி பலம் ஒன்றுக்கு(35கி), செம்பருத்தி இலைச் சாறு பலம் ஆறு (210கி) விட்டு வெயிலில் காலை முதல் மாலை வரை வைக்கவும்.இவ்விதம் ஆறு நாள் தினமும் சாறுவிடாமல் உலர்த்தி வந்து ஏழாம் நாள் இரண்டு நாட்கள் சாறுவிடாமல் வெயிலில் உலர்த்தவும். பிறகு வெள்ளாட்டு நீரிலும், செந்நிறப் பசுவின் நீரிலும் முன்போலவே செய்யவும். முடிவில் முள்ளங்கிச் சாறு பலம் ஆறு(210கி) வீதம் பத்து நாள் வரை விட்டு, மேற்கூறிய வண்ணமே ஒவ்வொரு நாளும் வெயிலில் வைத்து,பிறகு இரண்டு நாள் சாறு விடாமலே உலர்த்தி, புதிய ஓட்டில் இட்டு, உமிநெருப்பில் ஈரஞ் சிறிதுமின்றி வறுத்து, தூய நீர்விட்டுக் கழுவி துணியால் ஈரத்தைப் போக்கி,வெயிலில் ஈரமின்றி உலர்த்தி எடுக்க சுத்தியாகும்[10]

- **தேரன் யமக வெண்பா**

2.கஞ்சாயிலைச் சாற்றில் தனித்தனியே ஏழுமுறை உருக்கி சாய்த்து எடுக்க சுத்தியாகும்.[16]

-சரக்கு சுத்தி செய்முறைகள்

3.குஞ்சு பொரித்த முட்டைத் தோல், தொட்டால் வாடி சமன் எடை சேர்த்து அரைத்து தாமிரம் மெல்லியதாய்த் தட்டி கவசஞ் செய்து சீலை மண் மூன்று செய்து பத்து எருவில் புடம் போடவும். இவ்வாறு மூன்று புடம் போடவும் சுத்தியாகும். [16]

-சரக்கு சுத்தி செய்முறைகள்

4. தாயான வேதைக்குச் செம்புசுத்தி
சாத்துறேன் புலத்தியனே கேளுகேளு
வேயான புளிமோரில் பசுமஞ்சளிட்டு
விரைந்திடித்து பிழிந்துக்கொண்டு இதிலேசெம்பை
ஓயாம லுருக்கிவிடு பதினொன்றப்பா
உத்தமனே யிதன்பிறகு சாய்க்கக்கேளு
வாயான பூசணிக்காய் சாற்றினுள்ளே
வார்த்திடாய் பதினொன்று தரந்தான்பாரே
பாரப்பா வெண்ணொச்சி சாற்றினுள்ளே
பரிவாகச் சூடனையும் கரைத்துக்கொண்டு
நேரப்பா ஐயைந்து தரமும் சாய்க்க
நிறையான செம்பதுவும் சுத்தியாச்சு

-அகத்தியர் மகாதிராவகம்[17]

செம்பு ஒரு பலம் எடுத்துக் கொண்டு அதை புளித்தமோரில் பசுமஞ்சள் கிழங்கு போட்டு இடித்து பிழிந்த சாற்றில் பதினொன்றுதரம் உருக்கி சாய்க்கவும். பின் பூசணிக்காய் சாற்றினுள்ளே பதினொன்றுதரம் உருக்கி சாய்க்கவும்.அதன் பிறகு வெண்ணொச்சி சாற்றினுள்ளே சூடனை கரைத்துக்கொண்டு ஐயைந்து தரமும் சாய்க்க செம்பு சுத்தியாகும்.

5.
ஆமப்பா அறுகன் வேர் கீரிமூலி
அழகான மேனியது ரெண்டுங் கூட்டி
நாமப்ப அறுநீராலரைத் துருட்டி
நலம் பெறவே குகை பிடித்துச் செம்பை வைத்து
வாமப்பா சீலைமண் செய்துநீயும்
விள்ளாமற் கெஜபுடமே போட்டாயானாஞ்
சாமப்பா செம்பதுதான் சுத்தியாச்சு
கூஷணத்திலே செம்பதனை பதனம்பண்ணே.

- **நந்தீசர் கருக்கிடை 300**[18]

அறுகன் வேர் குப்பைமேனி இரண்டும் ஒன்றாய் கூட்டி அறுவகை நீரால் அரைத்து குகை பிடித்துச் அதற்குள் செம்பை வைத்து சீலைமண் ஏழு செய்து கெஜபுடம் போட்டு எடுக்க சுத்தியாகும்.

6. தாம்பிரம் குகையில்வைத்து ஊதும்போது மதியுப்பு ஒரு பலம் போட்டு உருக்கவும்.பின்னர் அதில் (அண்டமது பலம் 2, சாரம் பலம் 2,வெங்காரம்-1/2 பலம்) கல்வத்தில் பொடித்து பொடியை தூவ செம்பினிடன் ஊறல் போகும்.[19]

-பதினெண் சித்தர்கள் வைத்திய சில்லறை கோவை பாகம் 2

செம்புக் கட்டு:

காணுவது பிருதிவியு மப்புமிகச் சேரே
சேர்த்தந்த யிடைக்கிநிகர் தாளகமுங் கெந்தி
செம்மையுள்ள துருசுடனே சாரமொடு லிங்கம்
பார்த்தந்த ஏழுசரக்குங் கல்வமதி லிட்டுப்
பக்குவமா யாட்டுவது பழத்தினுட சாற்றால்
வார்த்துமிக நன்றாட்டி வட்டதுபோற் பண்ணி
மைந்தனே புடம்போடு சிவயோகப் படிக்குக்
காத்துமிகப் புடமாறி யெடுத்துமிகப் பார்த்தால்
கருவான எழுசரக்குங் கட்டிமிகப் போமே.
கட்டியதோர் சரக்கதனை யெடுத்தந்த யிடைக்குக்
களிம்பான செம்புருக்கி யதனிலிதைத் தாக்கு
திட்டமுடன் செம்பதுவுங் கட்டிமிகப் போகும்.

- **இராமதேவர் பரைஞான கேசரி**[20]

இந்துப்பும்,பாறையுப்பும் சமனாய் எடுத்துக் கொண்டு இதற்கு நிகர் தாளகம், கெந்தகம், துருசு, சாரம், லிங்கம் ஆகிய ஏழு சரக்குகளையும் கல்வத்திலிட்டுப் பழச்சாற்றால் அரைத்து அடைதட்டி காயவைத்து புடம் போட்டு புடமாறியெடுக்க ஏழு சரக்கும் கட்டியிருக்கும். அதனை எடுத்து எடைக்கு எடை செம்புருக்கு முகத்தில் கொடுக்க கட்டிப்போகும்.

பொதுகுணம்:

"தாம்பிரத்தாற் சோரி பித்தஞ் சந்நியகு வைகபம்
வீம்பார்பி லீகமந்தம் வெண்மேகந்-தேம்பழலை
சூதகநோய் புண்கிரந்தி தோடசுவா சங்கிருமி
தாதுநட்டங் கண்ணோய்போம் சாற்ற"

-பதார்த்த குண சிந்தாமணி[21]

செம்பினால் இரத்த பித்தம்,சந்நி,கல்லீரல் நோய், கப நோய், பீலிகம், அக்கினி மந்தம், வெண்மேகம், பித்தவழலை, சூதக நோய், புண், கிரந்தி, திரிதோட சுவாசம், கிருமி, கண்ணோய் முதலியன தீரும்.

எமனுக்கு உயிரை நீக்குந்தன்மை இருப்பதுபோலச் செம்பிற்கு குன்மத்தை விரைவில் போக்கக் கூடிய தன்மை இருத்தலின் இதற்குக் "குன்ம காலன்" என்னும் பெயரும் உண்டு.

"உற்பவமே தினியஞரா மனித வர்க்கம்
ஓகோகோ செம்பினன்மை யுரைக்கொணாதே
கற்பனைக ளல்லவடா மகனே குன்ம
காலனென்றால் வாதசந்நி கலங்கும் பாரே."[22]

-போகர் குறுந்திரட்டு 300

இவ்வுலகத்திலுள்ள மக்களுள் எவன் ஒருவன் செம்பில் பொருந்திய களிம்பை நீக்கும் வகையும் அதனைப் பற்பஞ் செய்யும் முறையும், பற்பத்தை உண்ணும் சிறந்த வகையும் அறிகின்றானோ, அவன் மிக்க பசியையும்,தேக பலத்தையும் தவத்தில் உயர்ந்த மிருகண்டுமகாரிஷி புத்திரனாகிய மார்க்கண்டேயனைப் போல ஆயுள் விர்த்தியையும் அடைவான்.

"உறுவங்குறும் பசியும்பெறும்
உரமும் பெறும் உரமாம்
மிருகண்டருள் தநயன்பெறு
மிகையும்பெறு குவரே;
வருசெம்புறு மலினந்தெறு
வகையும்பொடி வகையும்
பருகும்பெரு விதமுந்தெளி
பவரம்புவி மிசையே[9]

குறிப்பு: தாம்பிர பற்பத்தைக் கொள்ளுங்கால் பத்தியம் கண்டிப்பாய் இருக்கவேண்டுமென்பதைப் "பருகும் பெருவிதம் என்றனர்.[9]

செம்பு பற்பத்தின் அளவு:

"சுத்த வவுடதஞ் சொன்ன பிரயோகம்
வித்தள வாக நால் வேற்றுமை யாயினும்
உத்தம மாதி யுரைக்கு மிரண்டிரண்
டத்தனை யேதொகை யாகும் பதினாறே."

-(திருமூலர்)[23]

கடுகு, தினை, சாமை, கொள் இவற்றுள் ஒவ்வொன்றையும் நன்னான்கு கூறாக்கி அப்பங்கினளவாய் கொள்ளல் வேண்டும். இவை முறையே உத்தமம் ஆதியாய் அதமாதமம் இறுதியாய்ப் பதினாறு பாகம் என்றனர்.

மருந்துண்ணும் நாளளவு:

ஏழு நாள் உத்தமம், பதினான்கு நாள் மத்திமம், இருபத்தொரு நாள் அதமம், இருபத்தெட்டு நாள் அதமாதமம்.

செம்பு பற்பம் சரிவர நீறியதற்குசோதனை:

தாம்பிர பற்பம், காரீயம் சமவெடை சேர்த்து வெங்காரமும் சரி சேர்த்து அரைத்து கரியில் வைத்து பழுக்க ஊதி எடுக்க சரிவர நீராமலிருந்தால் அழகான செம்பு தங்கம் போல் வந்துவிடும்.

நீற்றினங்களின் குணங்கள்:

செம்பு நீறினால் குருராயனென்று பேர் பெறும்[13]

செம்பு பற்பத்தின் துணை மருந்தும், பிணி நீக்கமும்:

- நீர் - சேத்தும நோய்கள்
- வெந்நீர் - வாத நோய்கள்
- நெய் - பித்த நோய்கள்
- பசுவின் பால் - வல்லை நோய்
- வெண்ணெய் - தலை நோய்கள்
- சர்க்கரை - கைவலி
- துளசி சாறு - முலையில் வரும் சிலந்தி
- கருப்பஞ்சாறு - நடுக்குவதம்
- கற்றாழைச் சாறு - கீல்களில் நரம்பு சுருட்டி வலித்து மேனியில் வியர்வை உண்டாக்கும்.

"வல்லை முதலாய் வருமல வாதைக்கு
நல்லை வடும்பொடி நாட்டும் மனுபானம்
ஒல்லை உதகம் உரமாகு மாதொரு
குல்லை யிறுதி கொடுப்பதா யுண்மையே"

-திருமூலர்[23]

செம்பு பற்பத்திற்கு ஆகும் பொருட்கள்:

- நெய்
- வெண்ணெய்
- பால்
- அப்பம்
- அடை
- சர்க்கரை
- பூரி
- அவல்
- பொரி
- பயறு

- வாழைப்பழம்

செம்பு பற்பத்திற்கு ஆகாப்பொருள்கள்:

"புளியொன்று தவிர வெல்லாம் புசிக்கினு நவையு றாதை
யிளைபெரு நெருப்புக் கீர மில்லையென் பதுபோ லாகும்
அளைமுர்லி குசம் காடியாமலி யாடது பாகற்
சுளையர மகட்ப ழம்நெய் சுதைசருக் கரைமா நன்றே".

- எழுமிச்சம்பழம்
- மாம்பழம்
- மாதுளைபழம்
- கிச்சிலிப்பழம்
- நாரத்தம்பழம்
- நாவற்பழம்
- வெள்ளரிப்பழம்
- நெல்லிப்பழம்
- பனங்கள்
- தமரத்தங்காய்
- காய்சொரிக்கீரை
- பீர்க்கங்காய்
- இலந்தைப்பழம்
- விளாம்பழம்
- பெருங்களா
- தயிர்
- மோர்
- மொச்சை
- பாகற்காய்

"ஒவ்வுதேன் கிருதஞ் சர்க்கரை யிளநீ ரொவ்வொன் றிருவகைக்கனுபானம்
உளதிது வேனும் வெற்றிலை துளசி யுதகமு முயர்பிணிக் கமையும்
செவ்விய வளவு கடுகத னிரட்டி தினமிரு பொழுதினிற் றிலமே
சிந்தமீ னச்சங் கடிப்பகை காணந் திலமிசை யறினல மிகுமே".

-தேரையர் சேகரப்பா[24]

செம்பு பற்பத்தின் சிறப்பு:

"பற்பமென்றாற் றாம்பிரமே பற்ப மாகும்;
பாசுபத ம஼ஃதேயாம் பார்த்த பேர்க்கு
விற்பனபண் டிதத்தாலே கால கேய
வினைக்கூட்ட வெம்படையை வீக்கு மப்பா!"

(போகர் குறுந்திரட்டு 300)[22]

"செம்பி னடலை சிவாத்திர மாகுமே
நம்பியெய் வார்கள் நமனைவெல் வார்; பிணி
வெம்பி யகல விடாரியை நேர்பாயும்
வம்பியல் லார்பலன் வாய்க்கவல் லார்களே."

(திருமூலர் திருமந்திரம் கன்ம காண்டம் 1000)[23]

செம்புடனே பிறந்து இடையறா நட்புப் பூண்டு ஒழுகும் கன்மடம் என்னும் களிம்பும் வெப்பும் அற நீங்கி செம்பு நற்குணம் பெற்ற பற்பமயின், அது வேகத்தில் பாசுபதாஸ்திரத்தையும், வன்மையில் சஞ்சீவியாகிய பிராண பதார்த்ததையும், வாக்குத் திறத்தில் ஆதிக்கிய சிந்தாமணியையும், உபகாரத்தில் பயாதர சீமூதத்தையும், ஆக்ருஷணத்தில் காரூடாதி பிரயோகங்களையும் ஒக்கும். வசியாதி அறுபத்து நான்கு கலைக்கியான விருத்தியைப் பண்டிதர்களுக்கு எக்காலத்தும் அளிக்கும்

மேலும் செம்பினால் கண்ணோய்கள் தொண்ணாற்றாறும் தீரும் எனக் கூறப்பட்டுள்ளது காரணம் கண் தீக்கூற்றை உடைய உறுப்பாகும். செம்பும் மேற்படி பூதாமிசம் பொருந்தியதாதலின் ,செம்பு கண்ணுக்கு நட்பு பொருளாகின்றது. ஆதலின், இது நோய்களை அணுகவொட்டாமல் கண்ணைக் காக்கின்றது. இக்காரணத்தை முன்னிட்டே காசம் குத்தும் சத்திரமும், கண் மருந்துகள் இடுகின்ற அஞ்சனக்கோலும், மற்றைய சத்திரங்களும் சிறப்பாக செம்பினால் செய்யப்பட்டனவாய் இருக்கின்றன.

முசுமுசுக்கைச் சாறு, துளசி சாறு, சமன் கூட்டிப் புளியின் வித்தளவு எடுத்து, அதில் தாம்பிர பற்பத்தை எள் முனையளவு சேர்த்து உண்டால், மரத்தின் வேரைத் தோண்டியகற்றக் கிளை முதலியனபட்டுப் போவது போல் சாதலைத் தடுத்துவிடும்.

"முசுமு சுக்கையிலை துளவ பத்ரிரசம்
மொழியி வைகலவை செய்ததில்
முளையெ ளிற்சிறிது புளியின் வித்தளவில்
முழுக வைத்திதையு ணயிலவே
முதன்மி ருத்தையடு மதைய டக்கபமு
முரியு மற்றதுவு முரியுமே
முதல றக்கிளைக ளறலெ னப்பிணிகண்
முழுது மற்றுவிடு மிதுமெயே".

-தேரன் மருத்துவ பாரதம்[25]

தாம்பிர பற்பத்தை அருந்தியவர்களடையும் சிறப்பு:

"செம்பின் பொடியுண்டு தேக முரமுண்டு
செம்பின் வடிவுண்டு செய்தவங் கையுண்டு
செம்பின் முடிவெலாந் தெய்வத மாகவோர்
செம்பின் குலந்திகழ் கரதின மாகுமே".

- **திருமூலர்**[23]

செம்பின் நஞ்சுக் குறிகுணங்கள்:

"பிசகு பற்பமுறை யெனி லத்தினெறி
பிறழு மச்சமிகு மிருமலே
பெருகு விக்கலொடு தலைதி ருப்பிவரும்
பிரமைமி குத்துவரு மறல்கள் வாய்
பெருகி யக்குலரு முரமெ ரிச்சலுறும்
பிளிறல் மெத்தவரு மிவைகள் தீர்
பெலம ருத்துமுறை யுணர்ம ருத்துவர்கள்
பெலமு ரைப்பரிய தவர்செய்வார்".

-தேரன் யமகவெண்பா[10]

செம்பின் நஞ்சால், உடல் நன்னெறியின்னின்று மாறி அச்சத்தை விளைவிக்கும். இருமல், விக்கல், தலைதூக்க வொட்டாத மயக்கம், கள்ளை போல் ஒழுகாநின்ற வாய் நீர், மார்பு எரிச்சல், யானையின் பிளிறல் போன்ற ஒலி முதலிய குணங்கள் உண்டாகும்.

செம்பின் நஞ்சுக்கு தீர்வு:

"இவ்வகை புரிந்த தாம்பிர பற்பத்
தியற்கையை யென்சொல்வேன் பிசகின்
இடையறாக் களிம்பாற் கொல்லுவ ததுவே
எலுமிச்சை சுக்கினா லதுபோம்".

-தேரன் யமகவெண்பா[10][42]

எலுமிச்சை சாற்றில் சுக்குத் தூளை கலந்து உட்கொள்ள வேண்டும்.

செம்பு பற்ப தோஷம்:

கற்கண்டு, காட்டுதானிய அரிசி அல்லது கெத்தமல்லி இவை இரண்டையும் சமபாகமாய் எடுத்து ஜலத்தில்விட்டு அரைத்து மூன்றுநாள் காலை மாலை குடித்துவந்தால் அவ்விஷத்தினால் உண்டாகும் ரோகங்கள் குணமாகும்.

செம்பு சேரும் மருந்துகள்:

- தாமிரபூபதி குளிகை
- இரத்தினாதி மாத்திரை[26]

  (நாட்சென்ற கண்வியாதிகளை தீர்க்கும்)
- இலகு இரத்தினாதி மாத்திரை[26]

  (பிள்ளைப்பாலிலிழைத்து கண்ணிலிட படலவகைகள்,

  காசவகைகள்,பில்லவகைகள்,கண்புகைச்சல் தீரும்.)
- மகா இரத்தினாதி மாத்திரை[26]

  (கண்ணோய்கள் 96-ம் தீரும்)
- அமரஞ்சனம்[26]

  (கண்வியாதிகள் தீரும்)
- நீலகருடாஞ்சனம்[26]

  (கண்ணோய்கள் தீரும்)
- தாம்பிராதி மாத்திரை[26]

  (பிள்ளைப்பாலிலிழைத்து கண்ணிலிட நன்மை தரும்)
- பச்சை தாம்பிராதி மாத்திரை[26]

  (கண்ணிலுண்டாகும் வியாதிகள் தீரும்)
- மகா தாம்பிராதி மாத்திரை[26]

  (நீர்பில்லம்,வரட்பில்லம்,வெள்ளைபடலம், சதைபடலம்,

  இரத்தபடலம், வரியெழுச்சிப்படலம், நாகப்படலம்,

  அதிமாங்கிசபடலம்,கண்சிகப்பு, குத்தல்,கடுப்பு தீரும்.)
- அஞ்சனக்கோல்[26]

  (கண்ணோய்கள் தீரும்)
- விமலாதி மாத்திரை[26]

  (காசம் படலம் தீரும்)
- பஞ்சலோகாதி மாத்திரை[26]

  (பில்லம், படலம், காசம்,குந்தம் தீரும்)

- காச மாத்திரை[26]

  (கண்ணோய்கள் 96-ம் தீரும்)

- கனத்த தாம்பிராதி மாத்திரை

  (கண்ணோய்கள் எல்லாம் தீரும்)

- தாம்பிர கட்டு செந்தூரம்
- தாம்பிர களங்கு
- தாம்பிர சுண்ணம்
- தாம்பிர செந்தூரம்[27]
- பஞ்சீகரண செந்தூரம்[28]

COPPER

Copper:

- Copper is a chemical element with symbol Cu
- Atomic number 29.

It is a soft, malleable, and ductile metal with very high thermal and electrical conductivity. [29]

A freshly exposed surface of pure copper has a reddish-orange color.

Copper is used as a conductor of heat and electricity, as a building material, and as a constituent of various metal alloys, such as sterling silver used in jewellery, cupronickel used to make marine hardware and coins, and constantan used in strain gauges and thermocouples for temperature measurement.

Copper is essential to all living organisms as a trace dietary mineral because it is a key constituent of the respiratory enzyme complex cytochrome c oxidase. In mollusks and crustaceans, Copper is a constituent of the blood pigment hemocyanin, replaced by the iron-complexed hemoglobin in fish and other vertebrates. Copper is one of a few metallic elements with a natural colour other than grey or silver. Pure copper is orange-red and acquires a reddish tarnish when exposed to air.

Chemical:

Copper does not react with water, but it does slowly react with atmospheric oxygen to form a layer of brown-black copper oxide which, unlike the rust that forms on iron in moist air, protects the underlying metal from further corrosion. A green layer of verdigris (copper carbonate) can often be seen on old copper structures, such as the roofing of much older building. And the Statue of Liberty. Copper tarnishes when exposed to some sulphur compounds, with which it reacts to form various copper sulfides.

Biochemical functions:[30]

1. Copper is an essential constituent of several enzymes. These include cytochrome oxidase, catalase, tyrosinase, superoxide dismutase, monoamine oxidase, ascorbic

acid oxidase, ALA synthesis, phenol oxidase and uricase. Due to its presence in a wide variety of enzymes, Copper is involved in many metabolic reactions.

2. Copper is necessary for the synthesis of hemoglobin (Cu is a constituent of ALA synthase).

3. Lysyl oxidase (a copper-containing enzyme) is required for the conversion of certain lysine residues of collagen and elastin to allysine which is necessary for cross-linking these structural proteins.

4. Ceruloplasmin serves ass ferroxidase and is involved in the conversion of iron from Fe^{2+} To Fe^{3+} in which form iron (transferrin) is transported in plasma.

5. Copper is necessary for the synthesis of melanin and phospholipids.

6. Development of bone and nervous system (myelin) requires Cu.

7. Certain copper-containing non-enzymatic proteins have been identified, although their functions are not clearly known. These include hemocuprein (storage form in the liver),cerebrocuprin (in the brain) and hemocuprein (in RBC).

8. Hemocyanin, a copper-protein complex in invertebrates, functions like hemoglobin for O_2 transport.

Dietary requirements: [30]

- Adults - 2-3 mg/day
- Infants and children - 0.5-2 mg/day

Sources:

Liver, kidney, meat, egg yolk, cereals, nuts and green leafy vegetables. Milk is a poor source.

Dietary needs [31]

Copper is an essential trace element in plants and animals, but not all microorganisms. The human body contains copper at a level of about 1.4 to 2.1 mg per kg of body mass.

Copper is absorbed in the gut and then transported to the liver bound to albumin. After processing in the liver, copper is distributed to other tissues in a second phase, which involves the protein ceruloplasmin, carrying the majority of

copper in blood. Ceruloplasmin also carries the copper that is excreted in milk and is particularly well-absorbed as a copper source.

Copper in the body normally undergoes enterohepatic circulation (about 5 mg a day, vs. about 1 mg per day absorbed in the diet and excreted from the body), and the body is able to excrete some excess copper, if needed, via bile, which carries some copper out of the liver that is not then reabsorbed by the intestine.

Plasma copper: [30]

The copper concentration of plasma is about 100-200µg/dl. Most of this (95%) is tightly bound to ceruloplasmin while a small fraction (5%) is loosely held to albumin. The normal concentration of serum ceruloplasmin is 25-50 mg/dl. It contains about 0.34% copper (6-8 atoms of Cu per molecule, half in Cu^{2+} state and the other half in Cu^{+} state). Ceruloplasmin is not a transport protein since this copper is not readily exchangeable with other molecules. The RBC contains erythrocuprein (superoxide dismutase).

Effects of copper deficiency:[32]

1. Although iron absorption is not disturbed the release of iron into the plasma is prevented due to the decreased synthesis of ceruloplasmin. As a result, hypoferremia occurs which leads to the depressed synthesis of heme developing anaemia in severe deficiency of copper.
2. The experimental animals on a copper deficient diet lose weight and die.
3. In copper deficient lambs, low cytochrome oxidase activity results in neonatal ataxia.
4. Copper deficiency produces marked skeletal changes, osteoporosis, and spontaneous fractures.
5. Elastin formulation is impaired in the deficiency of copper. Because a copper-containing enzyme plays an important role in the connective tissue
6. Metabolism, especially in the oxidation of lysine into aldehyde group which is necessary for cross-linkage of the polypeptide chains of elastin and collagen.
7. Copper deficiency results in myocardial fibrosis in cows. It is suggested that reduction in cytochrome oxidase activity may lead to cardiac hypertrophy.

TOXICITY:

Copper in the blood and blood stream exists in two forms: bound to ceruloplasmin (85–95%), and the rest "free", loosely bound to albumin and small molecules.

Free copper normally reduces oxidative stress, as it is involved in the metabolic elimination of reactive oxygen species, such as with the superoxide radical through Cu-Zn dependent superoxide dismutase. Excessive free copper impairs zinc homeostasis, and vice versa, which in turn impairs antioxidant enzyme function, increasing oxidative stress. Chronically elevated levels of copper intake produce zinc deficiency. [33]

Nutritionally, there is a distinct difference between organic and inorganic copper, according to whether the copper ion is bound to an organic ligand. Organic copper, like that found in food, is a beneficial micronutrient needed for good health. Inorganic metallic copper, like that found in electrical wire, plumbing pipes, brass fittings, redox water filters, sheet metal, cooking utensils, jewelry, and pennies, is a neurotoxic heavy metal linked to physical and psychiatric symptoms on par with mercury and lead.

Symptoms:

Acute symptoms of copper poisoning

- Vomiting,
- Hematemesis (vomiting of blood),
- Hypotension (low blood pressure),
- Melena (black "tarry" feces),
- Coma,
- Jaundice (yellowish pigmentation of the skin),
- Gastrointestinal distress.
- Individuals with glucose-6-phosphate deficiency may be at increased risk of hematologic effects of copper. Hemolytic anemia resulting from the treatment of burns with copper compounds is infrequent.

Chronic symptoms of copper poisoning

Chronic (long-term) effects of copper exposure can damage the liver and kidneys.

Mammals have efficient mechanisms to regulate copper stores such that they are generally protected from excess dietary copper levels.

The diagnostic difficulties arise from the fact that many of the substances that protect us from excess copper perform important functions in our neurological and endocrine systems. When they are used to bind copper in the plasma, to prevent it from being absorbed in the tissues, their own function may go unfulfilled.

Such symptoms often include

- Mood swings,
- Irritability,
- Depression,
- Fatigue,
- Excitation,
- Difficulty focusing,
- Feeling out of control, etc.

To further complicate diagnosis, some symptoms of excess copper are similar to those of a copper deficit.

Toxicity in mammals includes a wide range of animals and effects such as

- Liver cirrhosis,
- Necrosis in kidneys and the brain,
- Gastrointestinal distress,
- Low blood pressure,
- Fetal mortality.

TREATMENT:

In cases of suspected copper poisoning, penicillamine is the drug of choice, and dimercaprol, a heavy metal chelating agent, is often administered. Vinegar is not recommended to be given, as it assists in solubilizing insoluble copper salts. The inflammatory symptoms are to be treated on general principles, as are the nervous ones. [34] There is some evidence that alpha-lipoic acid (ALA) may work as a milder chelate of tissue-bound copper. Alpha lipoic acid is also being researched for chelating other heavy metals, such as mercury.

COOKWARE:

Cookware in which copper is the main structural element (as opposed to copper clad, copper sandwiched or copper colored) is sometimes manufactured without a lining when intended to be used for any of a number of specific culinary tasks, such as preparing preserves or meringues.

Excepting for acute or chronic conditions, exposure to copper in cooking is generally considered harmless. Following Paracelsus, dosage makes the poison; as this pertains to copper "a defense mechanism has apparently evolved as a consequence of which toxicity in man is very unusual."

Acute exposure and attendant copper toxicity is possible when cooking or storing highly acidic foods in unlined copper vessels for extended periods, or by exposing foodstuffs to reactive copper salts (copper corrosion, or verdigris).

Continuous, small exposures of acidic foods to copper may also result in toxicity in cases where either surface area interaction potentials are significant, pH is exceptionally low and concentrated (in the case of cooking with, for example, vinegar or wine), or both, and insufficient time elapses between exposures for normal homeostatic elimination of excess copper.

BIRTH CONTROL:

Estrogen birth control pills may increase the amount of copper in humans but be not shown to increase absorption. Copper Intrauterine devices (IUDs) have been questioned anecdotally, with people claiming copper toxicity, but there is currently no scientific evidence to substantiate this claim. Estrogen increases the absorption of copper, making women more likely to carry excess copper even when no birth controlis used.

There are conditions in which an individual's copper metabolism is compromised to such an extent that birth control may cause an issue with copper accumulation.

They include toxicity or just increased copper from other sources, as well as the increased copper level of the individual's mother via the placenta before birth. The two hormones commonly used in birth control, estrogen and progestin, protect from each other's complications, so a combination method may work best. At least when existing imbalances have been treated.

PATHOPHYSIOLOGY:

Indian childhood cirrhosis

One manifestation of copper toxicity, cirrhosis of the liver in children (Indian childhood cirrhosis), has been linked to boiling milk in copper cookware. The Merck Manual states recent studies suggest that a genetic defect is associated with this particular cirrhosis.[38]

Wilson's disease

An inherited condition called Wilson's disease causes the body to retain copper since it is not excreted by the liver into the bile. This disease, if untreated, can lead to brain and liver damage, and bis-choline tetrathiomolybdate is under investigation as a therapy for Wilson's disease.

Alzheimer's disease [32]

Elevated free copper levels exist in Alzheimer's disease, which has been hypothesized to be linked to inorganic copper consumption. Copper and zinc are known to bind to amyloid beta proteins in Alzheimer's disease. This bound form is thought to mediate the production of reactive oxygen species in the brain.

AQUATIC LIFE:

Too much copper in water may damage marine and freshwater organisms such as fish and mollusks. Fish species vary in their sensitivity to copper, with the LD50 for 96-h exposure to copper sulfate reported being in the order of 58 mg per liter for Tilapia and 70 mg per liter for catfish. The chronic effect of sublethal concentrations of copper on fish and other creatures is damage to gills, liver, kidneys and the nervous system. It also interferes with the sense of smell in fish, thus preventing them from choosing good mates or finding their way to mating areas.

4. MATERIALS AND METHODS

4.1 Selection of the test drug:

The test drug Tambira parpam was selected for the evaluation of toxicity studies in Wistar albino rats

Ingredients of Tambira parpam:

- Copper
- Vettrilai charu (*Piper betel* juice)
- Munnaiilai charu (*Premna corymbosa* juice)
- Ilai Kalli charu (*Euphorbia ligularia* juice)
- Vanniilai charu (*Prosopis spicigera* juice)
- Punnai oil (*Calophyllum inophyllum oil)*
- Kalapai kizhangu oil (*Gloriza suberba oil*)

Procurement of the Raw drugs:

Thurusu (Copper sulphate) was procured from a reputed country shop in Chennai. The following herbal drugs such as vettriilai (*Piper betel*) procured from Tambaram market, Chennai. Then Munnaiilai (*Premna corymbosa*) Ilai kalli(*Euphorbia ligularia)* Vanniilai(*Prosopis spicigera)* collected from NIS Campus Chennai.

Identification and Authentication of the raw drug:

The Herbal drugs were Identified and authenticated by Botanist, NIS Tambaram Sanatorium Chennai (Certificate No: NISMB2332016), The Metal drug was Identified and authenticated by pharmacologist in Siddha Central Research Institute (SCRI) Arumbakkam Chennai.

The purification process of Copper:

1.Copper powder
2.Leaves juice of Red Cotton Tree (*Gossypium arboretum*)
3.Goat's urine
4.Red cow's urine
5.Garden radish juice (*Raphanus sativus*)

Copper powder is soaked for 6 hours in the leaves juice of red cotton tree (*Gossypium arboretum*) and insolation for six days, adding fresh juice every day.

A seventh and eighth day, it is insolation without adding the juice until the moisture evaporates. This procedure is repeated with goat's urine and red colored cow's urine. Finally, it is soaked in the juice of radish (*Raphanus sativus*) and insolated for ten days by adding fresh juice every day. It is then isolated for two more days without adding juice and then place d in a fresh frying pan and roasted in husk flame until moisture evaporates.Then it is washed in clean water and then drug was placed in a cotton cloth for removing moisture content and insolated for obtaining purified copper.

NOTE: Although it is stated that, it should be allowed to dry between each juice, if the watery contents does not absorb and dried, it should be placed in the daylight and then processed in the next juice in the sunlight which is called "Sooriya pudam" by which the drug gets efficacy through the seven colour present in the sunlight.

Gossypium arboretum ***Raphanus sativus***

PREPARATION:

35g purified copper was placed in kalvam and it was ground with the following juices, Vettrilai charu for 16 days, Munnaiilai charu for 12 days, Ilaikalli charu for 8 days, Vanniilai charu for 4 days. Then the grinding substance is made into a cake and dries it. After that, it was subjected to the pudam process.

In this above process, only 4 part out of 6 is turned into parpam. Again the parpam was treated with punnai oil and Kalapai kizhangu oil by the churukku process. After

that whole portion became parpam, then it was placed in dew. The above prepared Tambira parpam was kept in an air tight glass container.

Indication:

- Earl kuruku veekam(hepatomegaly)
- Nadukuvatham (Parkinson's diseases)
- Maarpu selanthe (Breast tumor)
- Yonni putru (vaginal cancer)
- Gunman (peptic ulcer)
- Pathavanmegam (filariasis)
- Naatpatta thalaivali (chronic headache) etc

Adjuvant: Honey, ghee, milk, hot water, butter.

Dose: 1.5 mg twice a day for 7 days

Purified Copper

Piper betel

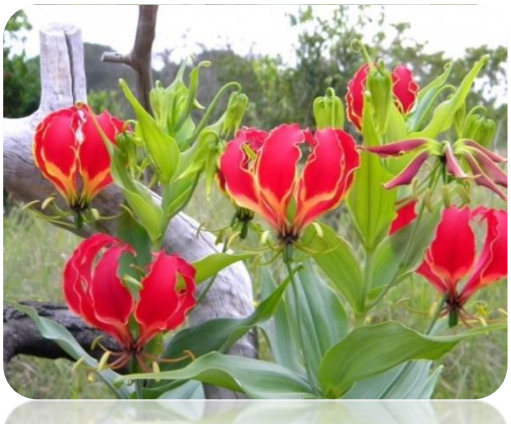
Premna corymbosa

Euphorbia ligularia

Prosopis spicigera

Calophyllum inophyllum

Gloriza suberba



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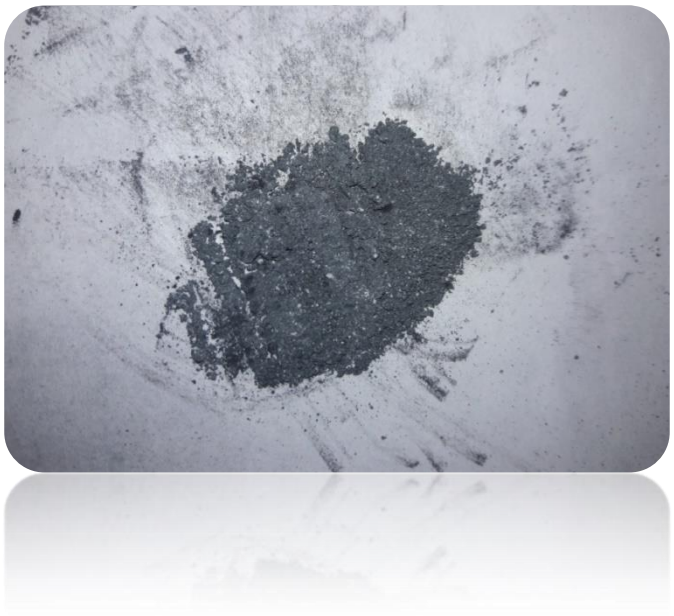

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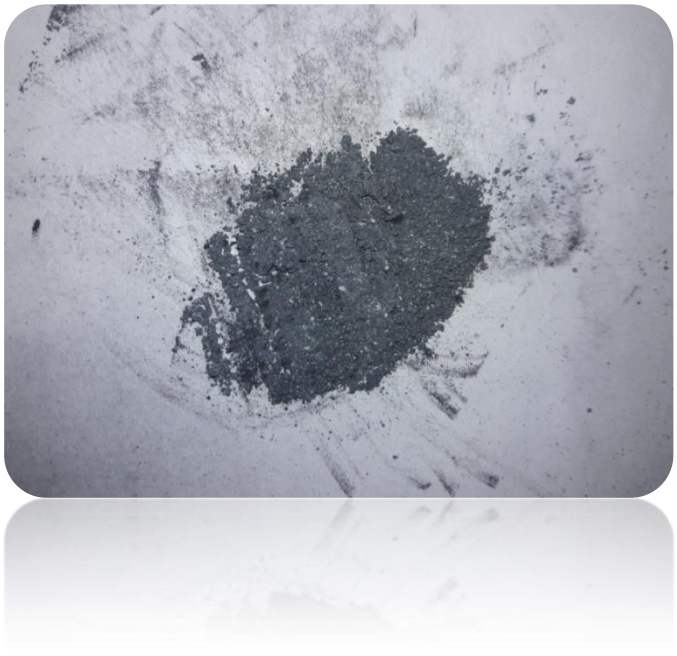

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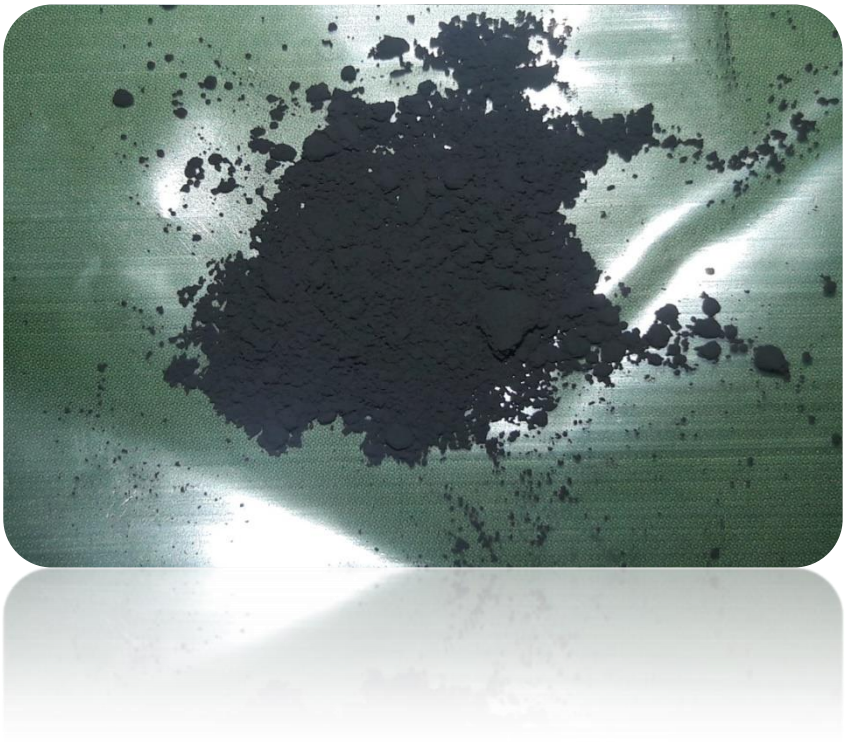

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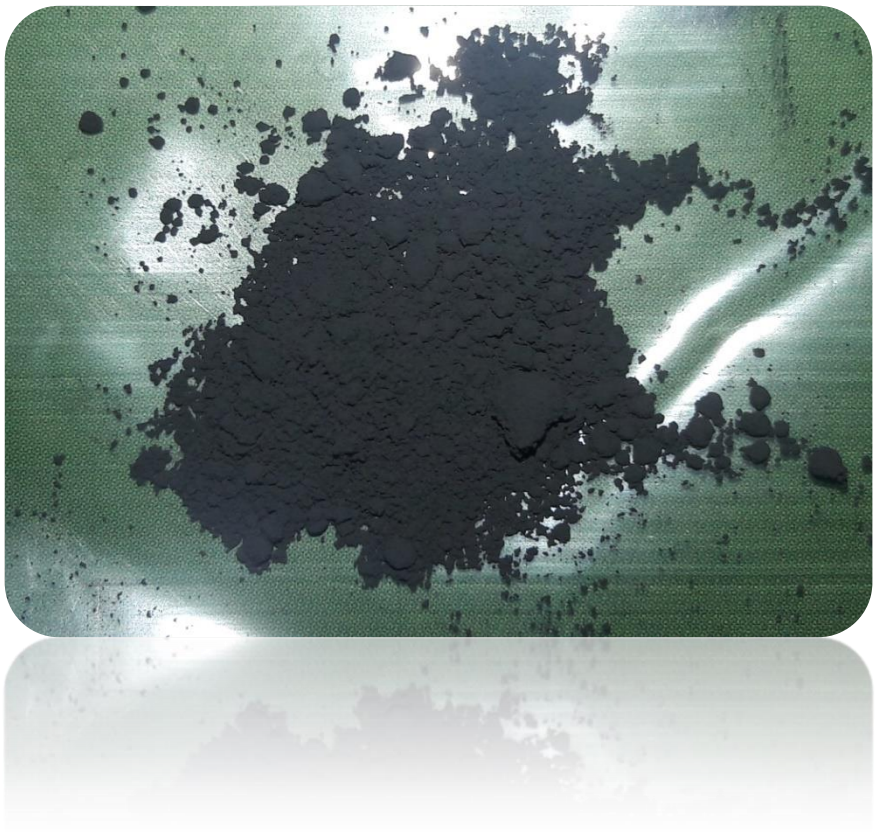


செம்பு பற்பம்-புன்னை விதை தைலம் மற்றும் கலப்பைக் கிழங்கு தைலம் சுருக்கு கொடுத்தப்பின்

ANALYTICAL STUDY OF TAMBIRA PARPAM:

The Tambira parpam was subjected to following analytical studies like physicochemical analysis, Biochemical Analysis and Quantitative analysis by using sophisticated instruments.

4.2 QUALITATIVE ANALYSIS

The Tambira parpam was studied by physicochemical parameters. This study was done at The Tamil Nadu Dr. M.G.R. Medical University No.69, Anna Salai, Guindy, Chennai-600032, and Bureau Veritas Consumer Products Services (India) Pvt.Limited Chennai.

A. PHYSICO-CHEMICAL PROPERTIES

1. Moisture Content:

An accurately weighed 3g of Tambira parpam was taken in a tarred glass bottle. The crude drug was heated at 105^0C in an oven till a constant weight. Percentage moisture content of the sample was calculated with reference to the shade dried material.[35]

Calculation:

$$\text{Percentage of loss on drying at } 105^{o}C = \frac{\text{Loss in weight of test drug}}{\text{Weight of test drug taken}} \times 100$$

2. Determination of total ash:

Weighed accurately 2g of Tambira parpam was added in the crucible at a temperature 500-600^0C in a muffle furnace till carbon- free ash was obtained. It was calculated with reference to the air- dried drug. [35]

Calculation:

$$\text{Percentage of total ash} = \frac{\text{Weight of the ash}}{\text{Weight of test drug taken}} \times 100$$

3. **Determination of acid insoluble ash:**

Ash above obtained, was boiled for 5min with 25ml of 1M Hydrochloric acid and filtered using an ashless filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffler furnace. The percentage of acid insoluble as was calculated with reference to the air-dried powdered drug. [35]

Calculation:

$$\text{Percentage of acid-insoluble ash} = \frac{\text{Weight of the acid-insoluble residue}}{\text{Weight of test drug taken}} \times 100$$

3. Determination of water soluble ash:

Total ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ashless filter paper was washed with hot water and ignited for 15min at a temperature not exceeding 450^0C in a muffle furnace. The difference in weight of ash and weight of water. [35]

4. Determination of water soluble Extractive:

1gm of air dried drug, coarsely powered Tambira parpam was macerated with 100ml of distilled water in a closed flask for twenty-four hours shaking frequently. The solution was filtrated and 25 ml of filtrate was evaporated in a tarred flat bottom shallow dish, further dried at 100^0 C and weighted. The percentage of water soluble extractive was calculated with reference to the air- dried drugs. [35]

Calculation:

$$\text{Percentage of water soluble extract} = \frac{\text{Weight of the extract}}{\text{Weight of sample taken}} \times \frac{100}{25} \times 100$$

6. **Determination of alcohol soluble extractive:**

1 gm. of air dried drugs, coarsely powdered Tambira parpam was macerated with 100 ml. alcohol in closed flask for 24 hrs. With frequent shaking. It was filtered rapidly taking precaution against loss of alcohol. 25ml of the filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100^0C and weighted. The percentage of alcohol soluble extractive was calculated with reference to air dried drug. [35]

Calculation:

$$\text{Percentage of alcohol soluble extract} = \frac{\text{Weight of the extract}}{\text{Weight of sample taken}} \times \frac{100}{25} \times 100$$

5. Determination of pH

The pH, of the Tambira parpam was estimated as per the method prescribed in the Indian standard (IS) APHA [36] 4500 H+A, B. The procedure was done at Bureau Veritas; Chennai 32.One gram of the test drug was taken into a 100ml graduated cylinder containing about 50 ml of water. The cylinder was shaken vigorously for two minutes and the suspension was allowed to settle for hour at 25ºC to 27ºC, then 25 ml of the clear aqueous solution was transferred into a 50 ml beaker and tested for pH using digital pH meter

B. CHEMICAL EVALUATION

Experimental procedure:

5 g of Tambira parpam was taken in a 250 ml of clean beaker and 50ml of distilled water was added to it. Then it was boiled well for about 10 min. Then it is allowed to cool and filtered into a 100 ml volumetric flask and made up to 100 ml with distilled water. This preparation is used for the qualitative analysis of acidic/ basic radicals and biochemical constituents in it. The biochemical analysis of Tambira parpam was done at Biochemistry Lab, National Institute of Siddha, Chennai-47.

A preliminary test for Copper, Sodium, Silicate and Carbonate:

S.No	EXPERIMENT	OBSERVATION	INFERENCE
1.	Appearance of sample	Dark grey in colour	
2.	**Test for Silicate:** a. A little (500mg) of the sample is shaken well with distilled water. b. A little(500mg) of the sample is shaken well with con. HCl/Con. H_2So_4	Sparingly not soluble	Absence of Silicate
3.	**Action of Heat:** A small amount (500mg) of the sample is taken in a dry test tube and heated gently at first and then strong.	White fumes evolved	Presence of Carbonate
4.	**Flame Test:** A small amount (500mg) of the sample is made into a paste with con. HCl in a watch glass and introduced into a non-luminous part of the Bunsen flame.	Bluish green flame appeared	Presence of Copper
5.	**Ash Test:** A filter paper is soaked in a mixture of sample and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited.	No Yellow colour flame appeared	Absence of Sodium

Test For Acid Radicals

S.No	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Test For Sulphate:** 2ml of the above-prepared extract was taken in a test tube and 2ml of 4% dil. ammonium oxalate solution was added.	Presence of Cloudy appearance	Sulphate present
2.	**Test For Chloride:** 2ml of the above-prepared extracts was added with 2ml of dil-HNO_3 until the effervescence ceases off. Then 2 ml of silver nitrate solution was added.	Presence of Cloudy appearance	Chloride present
3.	**Test For Phosphate:** 2ml of the extract was treated with 2ml of con.HNo3 and 2ml of dil. ammonium molybdate solution.	Absence of Yellow precipitate	Phosphate absent
4.	**Test For Carbonate:** 2ml of the extract was treated with 2ml dil. magnesium sulfate solution	Presence of Cloudy appearance	Carbonate Present
5.	**Test For Nitrate:** 1gm of the substance was heated with copper turning and concentrated H_2SO_4 and viewed the test tube vertically down.	Brown gas was not evolved	Nitrate absent
6.	**Test For Sulphide:** 1gm of the substance was treated with 2ml of the con. HCL	Rotten Egg Smelling gas was not evolved	Sulphide absent

7.	**Test For Fluoride & Oxalate:** 2ml of the extract was added with 2ml of dil. Acetic acid and 2ml dil. calcium chloride solution and heated**.**	Presence of Cloudy appearance	fluoride and oxalate Present
8.	**Test For Nitrite:** 3drops of the extract was placed on a filter paper, on that-2 drops of dil. acetic acid and 2 drops of dil. Benzidine solution was placed.	Characteristic changes not appeared	Nitrite absent

Test For Basic Radicals

1.	**Test For Lead:** 2ml of the extract was added with 2ml of dil. potassium iodine solution.	Yellow Precipitate was not obtained.	Lead absent
2.	**Test For Copper:** One pinch (50mg) of substance was made into a paste with con. HCl in a watch glass and introduced into the non-luminous part of the flame.	The blue colour precipitate formed.	Copper Present
3.	**Test For Aluminium:** In the 2ml of extract dil. sodium hydroxide was added in 5 drops to excess.	Yellow colour was not formed	Aluminium absent

4.	**Test For Iron:** a. To the 2ml of extract add 2ml of dil. ammonium solution b. To the 2ml of extract, 2ml thiocyanate solution and 2ml of con HNo3 is added	Absence of brown precipitate Red colour formed	Iron Present
5.	**Test For Zinc:** In 2ml of the extract dil. sodium hydroxide solution was added in 5 drops to excess and dil. Ammonium chloride was added.	White precipitate was not formed	Zinc absent
6.	**Test For Calcium:** 2ml of the extract was added with 2ml of 4% dil. ammonium oxalate solution	Cloudy appearance was formed	Calcium present
7.	**Test For Magnesium:** In 2ml of extract dil. sodium hydroxide solution was added in drops to excess.	white precipitate not formed	Magnesium absent
8.	**Test For Ammonium:** In 2ml of extract 1 ml of Nessler's reagent and excess of dil. sodium hydroxide solution was added.	Brown colour not formed	Ammonium absent
9.	**Test For Potassium:** A pinch (25mg) of substance was treated with 2ml of dil. sodium nitrite solution and then treated with 2ml of dil. cobalt nitrate in 30% dil. glacial acetic acid.	Yellowish precipitate formed	Potassium Present

10.	**Test For Sodium:** 2 pinches (50mg) of the substance was made into a paste by using HCl and introduced into the blue flame of Bunsen burner.	Yellow colour flame not appeared	Sodium absent
11.	**Test For Mercury:** 2ml of the extract was treated with 2ml of dil. sodium hydroxide solution.	Yellow precipitate not formed	Mercury absent
12.	**Test For Arsenic:** 2ml of the extract was treated with 2ml of dil. sodium hydroxide solution.	Brownish red precipitate not formed	Arsenic absent

Other constituents

1.	**Test For Starch:** 2ml of extract was treated with weak dil. iodine solution	Blue colour developed	Starch present
2.	**Test For Reducing Sugar:** 5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes.	The was no specific change in colour	Reducing sugar absent
3.	**Test For The Alkaloids**: a) 2ml of the extract is treated with 2ml of dil. Potassium iodide solution. b) 2ml of the extract is treated with 2ml of dil. picric acid.	Reddish brown precipitation not formed Yellow precipitation formed	Alkaloid Present

4.	**Test For Tannic Acid:** 2ml of the extract was treated with 2ml of dil. ferric chloride solution	Black precipitate not formed	Tannic acid absent
5.	**Test For Unsaturated Compound:** In the 2ml of extract 2ml of dil. Potassium permanganate solution was added.	Potassium permanganate not decolorized	unsaturated compounds Absent
6.	**Test For Amino Acid:** 2 drops of the extract were placed on a filter paper and dried well, and then 20ml of Biuret reagent was added to it.	Violet colour not developed	Amino acids absent

4.3 QUANTITATIVE ANALYSIS

Tambira parpam was analyzed in the presence of heavy metals by using ATOMIC ABSORPTION SPECTROMETER (AAS).This study was done at Asthagiri Herbal Research Foundation, 162-A, Perugudi Industrial Estate, Perungudi, Chennai-96

1. ATOMIC ABSORPTION SPECTROMETER (AAS)

ESTIMATION OF HEAVY METALS:

The procedure recommended for analysis of Heavy metals like Lead, Cadmium, Arsenic, and Mercury in WHO, 1998 and AOAC, 2005. and copper was analysed by Standard method.

INSTRUMENT DETAILS:

UV-Vis spectrometer AA240 series, UV 8500 Absorption Spectrometer(AAS) was used for the analysis. The operating parameters:

Instrument technique: UV Method

Wavelength (Lead) : 500 nm

Wavelength (Cadmium) : 228.8 nm

Wavelength (Mercury) : 253.7 nm

Wavelength (Arsenic) : 193.7 nm

Wavelength (Copper) : 324.8 nm

The hollow cathode lamp for Hg, As, Pb, Cd, and Cu were used as a light source to provide wavelength for the elements to be determined.

2. **Thermogravimetric analysis**

Thermogravimetric analysis or thermal gravimetric analysis (TGA) is a method of thermal analysis in which changes in physical and chemical properties of materials are measured as a function of increasing temperature (with constant heating rate), or as a function of time (with constant temperature and or constant mass loss).

TGA can provide information about physical phenomena, such as second-order phase transitions, including vaporization, sublimation, absorption, and desorption. Likewise, TGA can provide information about chemical phenomena including chemisorptions, desolvation (especially dehydration), decomposition, and solid-gas reactions (e.g., oxidation or reduction).[37]

TGA is commonly used to determine selected characteristics of materials that exhibit either mass loss or gain due to decomposition, oxidation, or loss of volatiles (such as moisture).

Common applications of TGA :[38]

(1) materials characterization through analysis of characteristic decomposition patterns.

(2) studies of degradation mechanisms and reaction kinetics, (3) determination of organic content in a sample.

(4) determination of inorganic (e.g. ash) content in a sample, which may be useful for corroborating predicted material structures or simply used as a chemical analysis. It is an especially useful technique for the study of polymeric materials, including thermoplastics, thermosets, elastomers, composites,

3.Fourier transform infrared spectroscopy (FTIR)

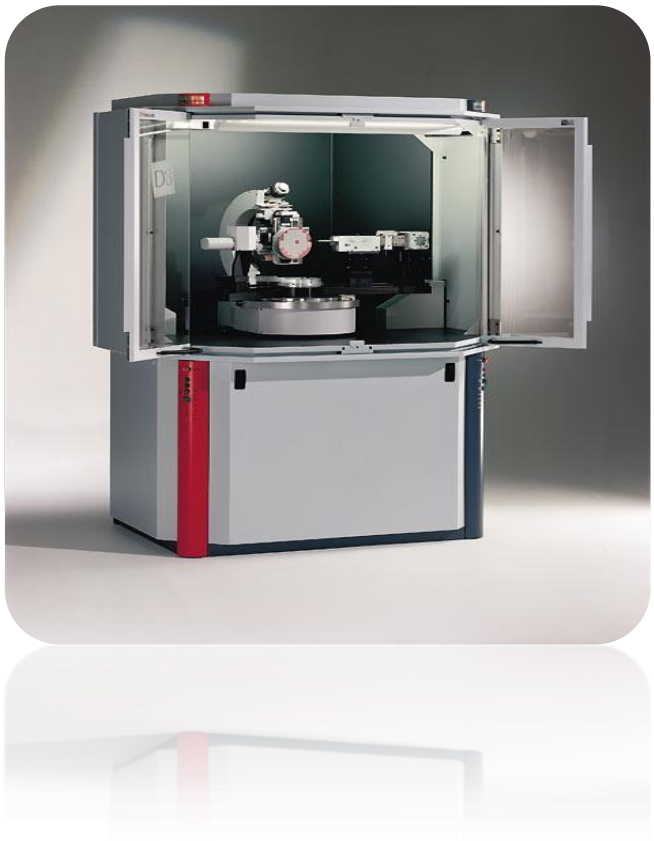
Fourier transform infrared spectroscopy (**FTIR**) is a technique which is used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time.

The term Fourier transform infrared spectroscopy originates from the fact that a Fourier transform (a mathematical process) is required to convert the raw data into the actual spectrum. For other uses of this kind of technique, see Fourier transform spectroscopy.

The standard method to prepare a solid sample for FTIR spectrometer is to use KBr. About 2 mg of Tambira parpam and 200 mg KBr are dried and ground. The particle size should be unified and less than two micrometers. Then, the mixture is squeezed to form transparent disc which can be measured directly. For liquids with a high boiling point or viscous solutions, it can be added in between two NACL pellets. Then

the sample is fixed in the cell by skews and measured. For a volatile liquid sample, it is dissolved in CS_2 or CCL_4 to form 10% solution. Then the solutions are injected into a liquid cell for measurement. Gas sample needs to be measured in a gas cell with two KBr windows on each side. That gas cell should first be vacuumed. Then the sample can be introduced into the gas cell for measurement.

4.X-ray Powder Diffraction (XRD)



X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analysed material is finely ground, homogenized, and average bulk composition is determined.

Crystalline substances act as three-dimensional diffraction gratings for X-ray wavelengths similar to the spacing of planes in a crystal lattice. X-ray diffraction is now a common technique for the study of crystal structures and atomic spacing.X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy Bragg's Law ($n\lambda=2d \sin \theta$). This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample.

These diffracted X-rays are then detected, processed and counted. By scanning the sample through a range of 2θangles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material. Conversion of the diffraction peaks to d-spacing allows identification of the mineral because each mineral has a set of unique d-spacings. Typically, this is achieved by comparison of d-spacing with standard reference patterns.

All diffraction methods are based on the generation of X-rays in an X-ray tube. These X-rays are directed at the sample, and the diffracted rays are collected. A key component of all diffraction is the angle between the incident and diffracted rays. Powder and single crystal diffraction vary in instrumentation beyond this.

5.SCANNED ELECTRON MICROSCOPY(SEM)

A SEM is essentially a high magnification microscope, which uses a focused scanned electron beam to produce images of the sample, both top-down and, with the necessary sample preparation, crosses sections. The primary electron beam interacts with the sample in a number of key ways:-

- A primary electron generates low energy secondary electron, which tends to emphasize the topographic nature of the specimen.
- A primary electron can be backscattered which produces images with a high degree of atomic number (Z) contrast.
- Ionized atoms can relax by electron shell-to-shell transitions, which lead to either X-ray emission or Auger electron ejection. The X-ray emitted are characteristic of the elements in the top few μm of the sample.

The SEM is carried out by using FEI-Quanta FEG 200-High Resolution Instrument.

Resolution: 1.2 nm gold particle separation on a carbon substrate

Magnification: From a min of 12x to greater than 1,00,000X

Application: To evaluate grain size, particle size distributions, material homogeneity and intermetallic distributions.

4.4 TOXICITY STUDIES OF TAMBIRA PARPAM

To evaluate the safety profile of Tambira parpam acute and subacute toxicity study carried out as followed

Principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of animals and the study design. Institutional Animal Ethical Committee approval number: (IAEC). (NIS/IAEC/I/2016/09 dated 12.2.2016) for acute toxicity study and (NIS/IAEC/II/2016/06 dated 28.3.2016) for repeated dose 28-day oral toxicity study.

1. ACUTE TOXICITY STUDY OF TAMBIRA PARPAM[7]

Experimental Animals:

Species	:	Wistar Albino Rats
Sex	:	Female
Age/weight	:	6 weeks/140-160g b.wt
Acclimatization Period	:	7 days prior to dosing
Housing	:	Polypropylene cages with bedding with Husk
Husbandry	:	12-h light/12-h dark cycle/ Room temperature 22°C ± 3°C and Relative humidity 30–70%
Feed and Water	:	Rodent pelleted feed RO purified water *ad libitum*
Identification	:	Animals will be kept in Polypropylene cages and Numbered

Experimentation Details of Acute Toxicity Study:

Groups/Treatment regimen	:	Grouped by randomisation
Test Guideline	:	OECD-423
Length of exposure to test substance	:	Once single dose
No of Animals	:	3 Female/ group
Control group	:	Vehicle (honey)
Test groups	:	Tambira parpam 5,50,300,2000 mg/kg. b.wt

The Female Albino Rats of weighing 150-200g were obtained from authorized animal breeders of the animal laboratory in TANUVAS, Madhavaram, and Chennai and stocked in the animal house at National Institute of Siddha, Chennai. Animals

were housed in a cage at 22°C ±3°C and relative humidity 30–70% and have free access to standard rat pellet diet (Sai Meera Foods Pvt. Ltd., Bangalore). The animals were treated with Tambira parpam by oral route for one day and monitored for behavioral parameters for the first 4 hours (1/2 hr, 1hr,2 hr, 3 hr,4 hr) after drug administration. Body weight of the animal will be monitored at weekly intervals. The animals that the die within this period will be subjected to necropsy. Remaining animals will be weighed and sacrificed under the injection of Pentothal Sodium on the 15th day of the Study period. The toxicological effect was assessed on the basis of mortality.

Preparation of Test Drug Doses:

Groups	No. of Rat
Group I: Vehicle control (honey)	3Female
Group II : test drug– 5mg/kg b.wt	3 Female
Group III: test drug – 50 mg/kg b.wt	3 Female
Group IV: test drug – 300 mg/kg b.wt	3 Female
Group V: test drug – 2000 mg/kg b.wt	3 Female

Route of administration

Oral route was selected because it is the normal route of clinical administration.

Administration of Dose

The animals were fasted (only food was withheld) for 12hrs and weighed prior to dosing. Three animals were used for each step. A single dose of the solution (5, 50, 300,2000mg/kg) was consecutively administered by oral gavage using intubation cannula. The food was withheld for another 4hrs after dosing and administration of the drug. As per the guideline, the starting dose level was taken as 5mg/kg body weight.

Observations:

Observations were made and recorded systematically and continuously observed after the substance administration as per the guidelines.

- ✓ ½ hour, 1 hour, 2 hours, 4 hours and up to 24 hours observation

- ✓ All rats were observed twice daily for 14 days
- ✓ Body weight were Calculated weekly once
- ✓ Feed & water intake were Calculated daily

Cage side observation

The animals were monitored for behavioral parameters like Alertness, Aggressiveness, piloerection, Grooming, Gripping, Touch Response, Motor Activity, Tremors, Convulsions, Muscle Spasm, Catatonia, Muscle relaxant, Hypnosis Analgesia, Lacrimation, Exophthalmos, Diarrhea, Writhing, Respiration, Mortality

Gross necropsy:

At the end of the 14 th day, all the animals were sacrificed by using the injection of Pentothal sodium Gross necropsy includes examinations of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents. Brain, eye, lungs, heart, spleen, liver, kidneys, adrenals, uterus, of all animals.

2. REPEATED DOSE 28-DAY ORAL TOXICITY STUDY OF TAMBIRA PARPAM[8]

Experimental Animals:

Species	:	Wistar Albino Rats
Sex	:	Male and Female
Age/weight at start of test	:	6 weeks/140-160g b.wt
Acclimatization Period	:	7 days prior to dosing
Housing	:	Polypropylene cages with bedding with Husk
Husbandry	:	12-h light/12-h dark cycle/ Room temperature 22°C ± 3°C and Relative humidity 30–70%
Feed and Water	:	Rodent pelleted feed RO purified water ad libitum
Identification	:	Animals will be kept in Polypropylene cages and Numbered

Experimentation Details of Repeated dose 28 days Toxicity Study:

Groups/Treatment regimen	:	Grouped by randomisation
Test Guideline	:	OECD-407
Length of exposure to test substance	:	28 days
No of Animals	:	3 Female+3 Male / group

Control group : Vehicle (honey)

Test groups : Tambira parpam (Low dose, Mid dose, High dose & Satellite group

The 30 Wistar albino rats of both sexes selected randomly. The animals were divided into five groups. Each group consists of 6 animals. The first group treated as vehicle control and second, third, four, fifth group were treated with Tambira parpam Low-dose (100 mg), Mid dose (200 mg), High-dose (400 mg) and satellite group (400 mg) respectively. The satellite group was included to determine delayed occurrence or recovery from toxic effects. The control animals were administered with honey as a vehicle.The other animals treated with **Tambira parpam** which was mixed with honey at the dose levels of Low dose 100mg/ kg b.wt, Mid dose200 mg/kg b.wt and High dose 400 mg/kg b.wt and Satellite group or to retrieval group 400mg/kg b.wt. For 28 days. The administration was given by oral, once daily for 28 consecutive days. The animals were observed the behavioural parameters for the study period. Body weight of the animal was being monitored at weekly intervals. Food & water intake were Calculated daily. All the animals were sacrificed at the end of the study (29 days) by using the injection of Pentothal Sodium and satellite group was sacrificed after 14 days of drug withdrawal by using an injection of Pentothal sodium. Blood was collected from the anesthetized animals from the Abdominal aorta for the following investigations like Haematology, Biochemical analysis. Gross pathological changes were monitored the animals and then the organs were studied by histopathological examination.

The doses (Low, Mid, High dose) were fixed from the result from the acute toxicity study

Groups	No. of Rats
Group I: Vehicle control (honey)	6(3M+ 3F)
GroupII:Test drug (Tambira parpam)- low dose (100mg/Kg b.wt)	6(3M + 3F)
GroupIII: Test drug(Tambira parpam) - Mid dose (200mg/Kg b.wt)	6(3M +3F)
GroupIV:Ttest drug(Tambira parpam) High dose (400mg/Kg b.wt)	6(3M +3F)
GroupV:Test drug(Tambira parpam)Satellite group(400mg/Kg b.wt)	6(3M+3F)

Observations:

Experimental animals were kept under observation throughout the course of study for the following

- ✓ All rats were observed twice daily for 28 days
- ✓ Body weight were Calculated weekly once
- ✓ Feed & water intake were Calculated daily

Cage side observation

The animals were monitored for behavioral parameters like, Alertness, Aggressiveness, piloerection, Grooming, Gripping, Touch Response, Motor Activity, Tremors, Convulsions, Muscle Spasm, Catatonia, Muscle relaxant, Hypnosis Analgesia, Lacrimation, Exophthalmos, Diarrhea, Writing, Respiration, Mortality.

Laboratory Investigations:

On the 29^{th} day, the animals were fasted overnight, then anesthetized to collect blood samples from the abdominal aorta in two tubes: one with EDTA for hematological parameters, another one without any anticoagulant and was centrifuged at 4000 rpm at 4°C for 10 minutes to obtain the serum for biochemical parameters.

***Hematological Investigations*:**

Blood samples of control and experimental rats were analyzed for haemoglobin (Hb), total red blood corpuscles (RBC), white blood corpuscles (WBC) count, Platelet, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), were calculated by auto analyzer.

***Biochemical Investigations*:**

Serum samples of control and experimental animals were analyzed for, Bilirubin, BUN, Creatinine, Triglyceride, Total Cholesterol, HDL, LDL, VLDL, using standard methods. Activities of glutamate oxaloacetate transaminase/

Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine aminotransferase (GPT/ALT) were estimated as per the colorimetric procedure.

Necropsy:

All the animals were sacrificed on the 29^{th} day and satellite group were sacrificed on after 14 days. Gross necropsy includes examinations of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents. Brain, eye, lungs, heart, spleen, liver, kidneys, adrenals, sex organs, of all animals were recorded.

Histopathology:

The organs included liver, kidneys, spleen, brain, heart, lungs and stomach of the animals were preserved, and they were subjected to histopathological examination.

Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of all the animals (low, mid, high) and satellite group were preserved and fixed in 10% formalin for 24 hrs. Samples were dehydrated in an auto technic and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at $50^{o}C$ and then in a cubical block of paraffin made by the "L" molds. It was followed by microtome and the slides were Prepared then stained with Haematoxylin-eosin.

Statistical analysis:

Findings such as body weight changes, food consumption, water intake, hematology and biochemical analysis were subjected to One-way ANOVA Dunnet's test using a computer software program followed by D *Graph Pad Instat-3*

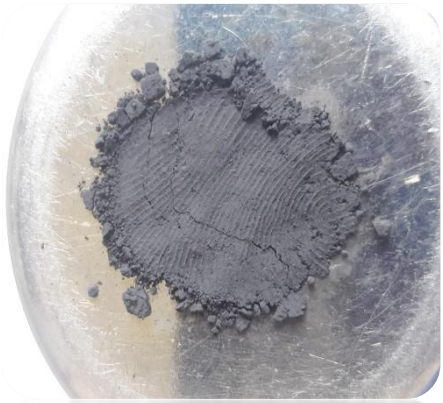
RESULT

Standardization of Parpam in Siddha Aspect

பற்பம் முடிந்த பின் அதனை சோதித்து பார்க்கும் முறைகள் சித்த மருத்துவத்தில் பின்வருமாறு.

- விரலின் ரேகை பற்பத்தில் பதிய வேண்டும்.
- நீரின் மேற்பரப்பில் பற்பத்தை போடும் போது நீரின் மேல் மிதக்க வேண்டும்.
- பற்பம் முடிந்தபின் அதனை கொல்லன் உலையில் மூசையிலிட்டு உருக்கும்போது பற்பம் நன்கு முடிந்திருப்பின் வைத்தது வைத்தது போன்றே இருக்கும். பற்பம் சரிவர முடியவில்லையெனில் முடியாத பாகம் உருகி சுத்த உலோகமாக வெளிப்படும்.

விரலின் ரேகை செம்பு பற்பத்தில்



நீரில் மிதக்கும் செம்பு பற்பம்

கொல்லன் உலை துருத்தி

மூசையில் உருக்கும் போது

5.1 QUALITATIVE ANALYSI S

PHYSICO-CHEMICAL ANALYSIS

Table-1: Colour, nature, and pH of Tambira parpam

S.no	Parameters	Results	Method of Testing
1.	Colour	Dark grey	By visual
2.	Odour	Odourless	Olfactory examination
3.	Solubility	• Soluble in honey&milk • Insoluble in water,acetone& ether	Qualitative
4.	Nature	Powder	By visual
5.	pH	8.97%	APHA 4500H+A,B

From Table 1, The Organoleptic characters shows that Tambira parpam is dark grey in colour and odourless powder form of drug with pH of 8.97. It is soluble in honey, milk and insoluble in water, acetone and ether.

Table-2: Physico-chemical properties of Tambira parpam

S.no	Parameters	Percentage
1	Moisture content	1.9160%
2	Total ash value	96.36%
3	Acid insoluble ash	22.84%
4	Water soluble ash	9.88%
5	Water soluble extraction	11.56%
6	Alcohol soluble extraction	1.84%

From Table 2, The Physico-chemical analysis of Tambira parpam explained in the parameters such as Moisture content, Total ash value, Acid insoluble ash, Water soluble ash, Water soluble extraction, Alcohol soluble extraction and pH are within the normal limits according to PLIM guidelines.

Table-3: Test for Basic radicals

S.no	Procedures	Tambira parpam
1.	Test for Ammonium	-
2.	Test for Sodium	-
3.	Test for Magnesium	-
4.	Test for Aluminium	-
5.	Test for Potassium	+
6.	Test for Calcium	+
7.	Test for Ferrous iron	+
8.	Test for Copper	+
9.	Test for Zinc	-
10.	Test for Arsenic	-
11.	Test for Mercury	-
12.	Test for Lead	-

From Table 3, The Biochemical analysis for basic radical reveals that Tambira parpam contains Potassium, Calcium, Iron, and Copper.

Table-4: Test for Acidic radicals

S.no	Procedures	Tambira parpam
1.	Test for Sulphate	+
2.	Test for Chloride	+
3.	Test for Phosphate	_
4.	Test for Flouride&Oxalate	+.
5.	Test for Nitrate	_

Table-5: Test for Acidic radicals

S.no	Procedures	Tambira parpam
1.	Test for Starch	+
2.	Test for Reducing sugar	_
3.	Test for Alkaloids	_
4.	Test for Amino Acids	_
5.	Test for Tannic acids	_
6.	Test for type of compounds	Oxyquinoline, epinephrine, Pyro catechol

From Table 4&5, The Biochemical analysis for acid radicals reveals that Tambira parpam contains Sulphate, Chloride, Fluoride, Oxalate, Starch, Oxyquinoline, epinephrine and Pyro catechol.

5.2 QUANTITATIVE ANALYSIS

A. ATOMIC ABSORPTION SPECTROSCOPY

Heavy metals content of Tambira parpam was analyzed by AAS this results Tabulated in Table 6.

Table-6: Analysis of Heavy Metals

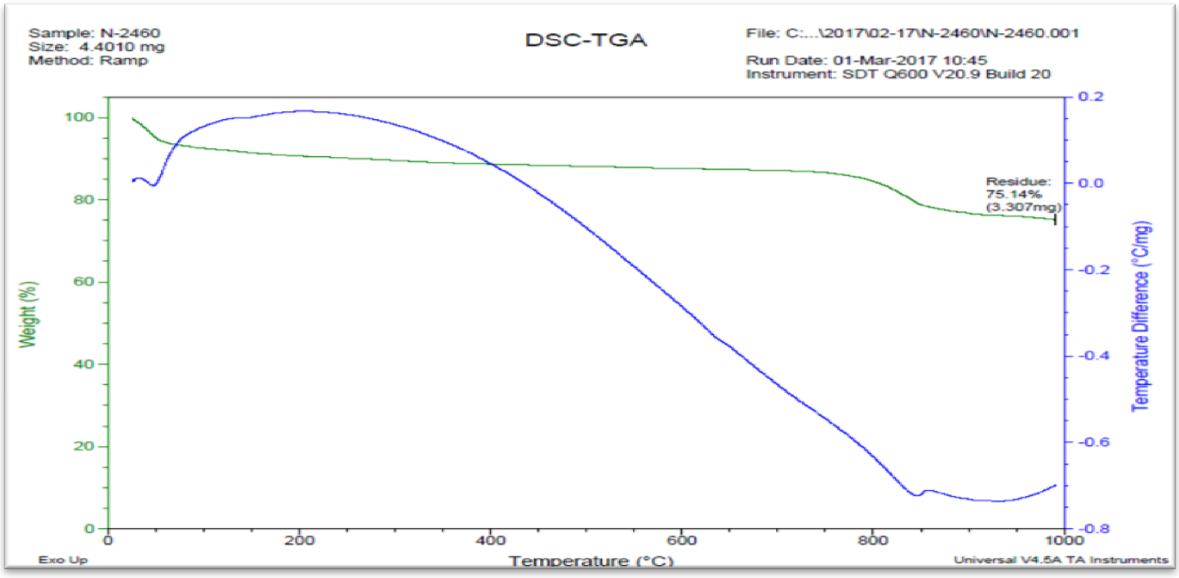
S.no	Name of the Element	Results	Permissible limit
1.	Lead	BDL	10 ppm(WHO)
2.	Cadmium	BDL	0.3 ppm(WHO)
3.	Mercury	BDL	1ppm(WHO)
4.	Arsenic	BDL	3ppm(WHO)
5.	Copper	11.139	-

BDL – Below Detection Limit

From Table 6, The Atomic Absorption Spectroscopy result shows that the heavy metals present in Tambira parpam were found to be within normal limits as per WHO, at the same time the Copper content of test drug is 11.139 ppm.

B.Thermogravimetry Analysis of Tambira parpam

Graph 1

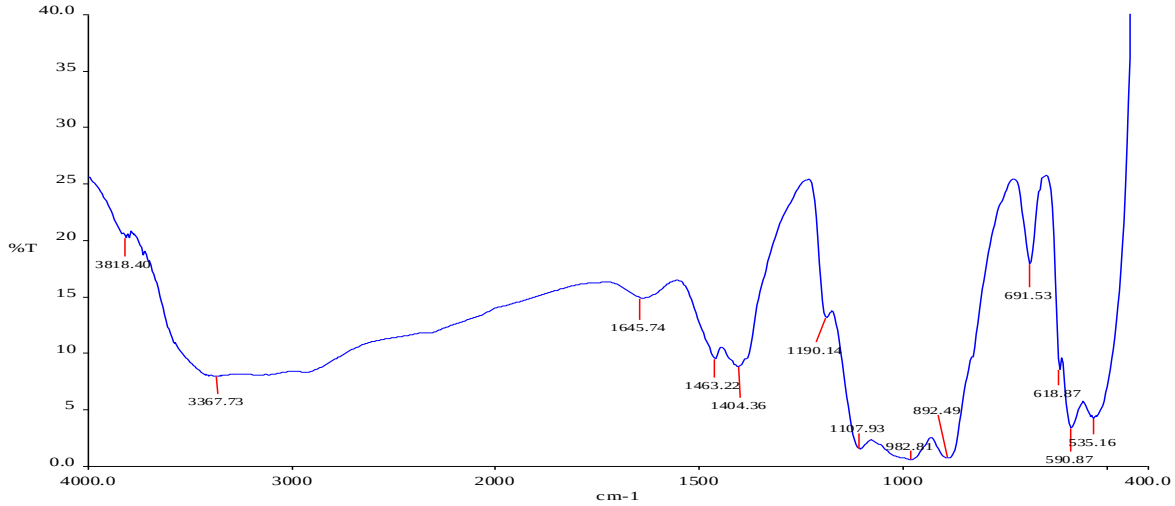


Thermogravimetry Analysis

Thermogravimetric analysis of Tambira Parpam carried out at the maximum of 1000^0C. The main objective of the study is to evaluate the decomposition and stability limit of the prepared formulation Tambira parpam. Prepared formulation Tambira parpam seems to be stable at the temperature varying from 50 ºC to 800 ºC with no variation in the residual weight. The point of decomposition begins when the temperature increases beyond 800 ºC. Sharp deletion curve observed from 800 ºC to 825 ºC and at this point suggested crystal transformation may be observed. Predicted denaturation may be due to atomic change at oxygen atom present within the sample. Weight of the final residual matter was observed as 3.307 mg with 75.14% of residual volume. The remaing portion (approximately 25%) contains organic compounds also.

C. FT-IR Analysis of Tambira Parpam

Graph 2

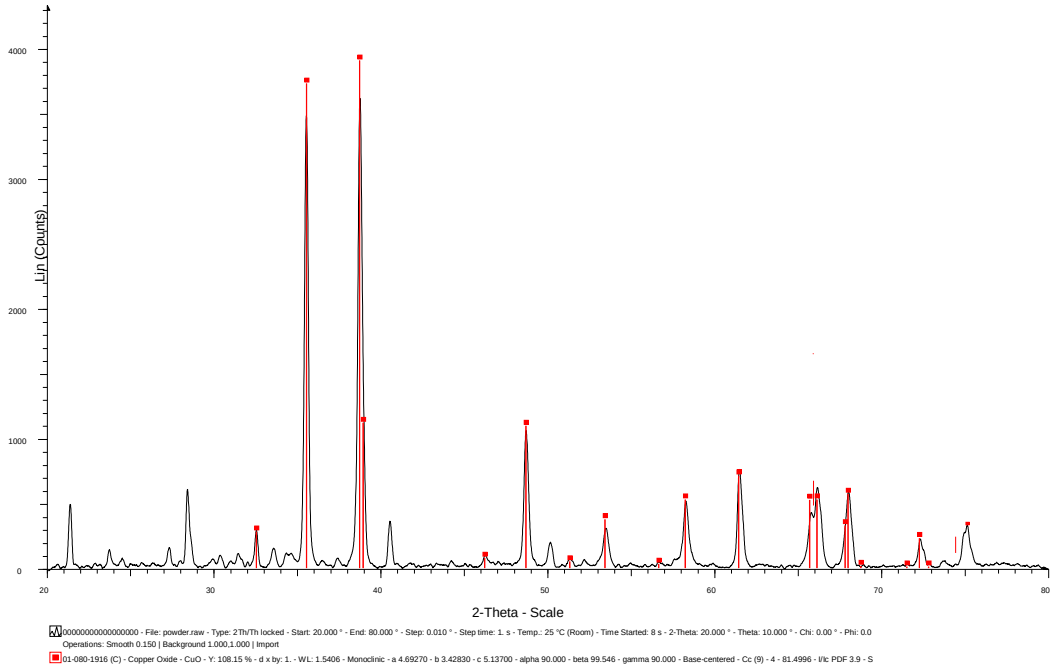


FTIR analysis of Tambira parpam

Infrared absorption pattern of CuO stretching was observed in the region of 523.16 cm−1 to 691.53 cm−1.Sharp absorption peak observed in the region of 590.87 cm−1 indicates the IR spectral pattern of CuO. Absorbance peak at 1107.93 cm−1 corresponds to CuO vibration due to metal cation. Broad absorption peak at 3367.73 cm−1 corresponds to O-H stretching which is bonded. Wide absorption peaks at 1645 cm−1 may be due to presence of primary amino group and also due to vibrational intensity of C=C group,1404 cm−1 corresponds to CH2 Bending, 1463 cm−1 corresponds to CH2 deformation.

D. X-ray diffraction pattern of Tambira parpam

Graph 4



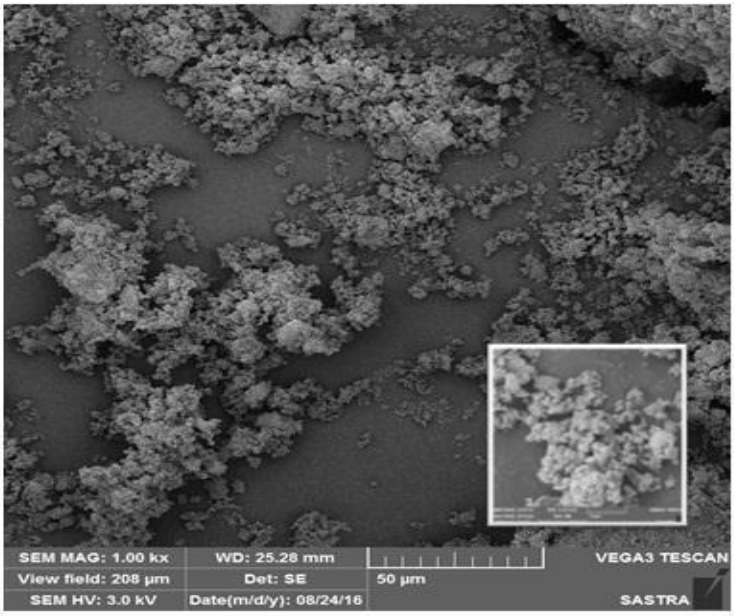
XRD Analysis

The X-ray diffraction pattern of the prepared formulation Tambira Parpam reveals the presence of a major peak with 2- Theta value of 38.719 which exactly matches to the ICDD (International Centre for Diffraction Data) 80- 1916. ICDD801916 corresponds to the crystalline pattern of copper oxide (CuO).Hence the reference matching material was confirmed as copper oxide (CuO). Major peaks observed in Tambira parpam with 2-theta values of 35.49 and their corresponding intensities were 3492.The major peak observed in the reference matching material was 38.68 with the intensity value of 999.The XRD pattern of the test (Tambira Parpam) exactly matches with the reference material CuO, which justifies the presence of stable and purified CuO in the formulation.

E. SCANNED ELECTRON MICROSCOPY

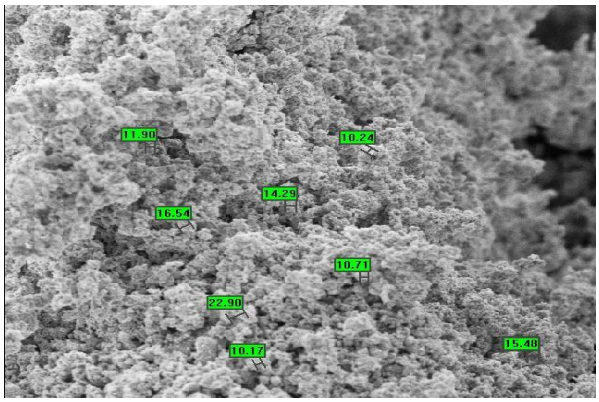
Determination of Particle size of Tambira parpam

Figure-1: SEM Image of Tambira parpam



SEM image of Tambira Parpam

Figure 2:



SEM image of Tambira Parpam

Results:

Particle Size : 10.17nm to 22.9 µm

Shape : Spherical

Surface : Smooth

Distribution : Evenly distribute

ACUTE TOXICITY STUDY

Table7. Behavioural Signs of Acute Toxicity Study of Tambira parpam[7]

No	Dose Mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	Control	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
2.	5	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
3.	50	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
4.	300	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
5.	2000	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

1.Alertness 2.Aggressiveness 3.pilo erection 4.Grooming 5.Gripping 6.Touch Response 7. Motor Activity 8. Tremors 9.Convulsions 10.Muscle Spasm 11.Catatonia 12.Muscle relaxant 13.Hypnosis 14.Analgesia 15.Lacrimation 16.Exophthalmos 17.Diarrhea 18.Writhing 19. Respiration 20.Mortality

\+ Presence of Activity

\- Absence of Activity

All the data were summarized in the form of (table-7) revealed that there was no abnormal signs and behavioural changes in all animals at the dose level of 5,50,300,2000 mg/kg body weight administered orally, during the study period.

There was no mortality observed after dosing of Tambira parpam upto 2000mg/kg body weight during the study period of 14 days. This indicates that the LD50 of Tambira parpam is more than 2000mg/kg b.wt.

There were no changes in skin and fur, eyes and mucous membranes of all animals. The eating, drinking habit, sleep pattern, locomotion were normal in all animals and no changes in body weight as compared to control group.

At the end of the 14 ^{the} day, necropsy was performed and there was no abnormality seen in test groups as compared to control group during the examination.

28 Day Repeated Dose Oral Toxicity Study

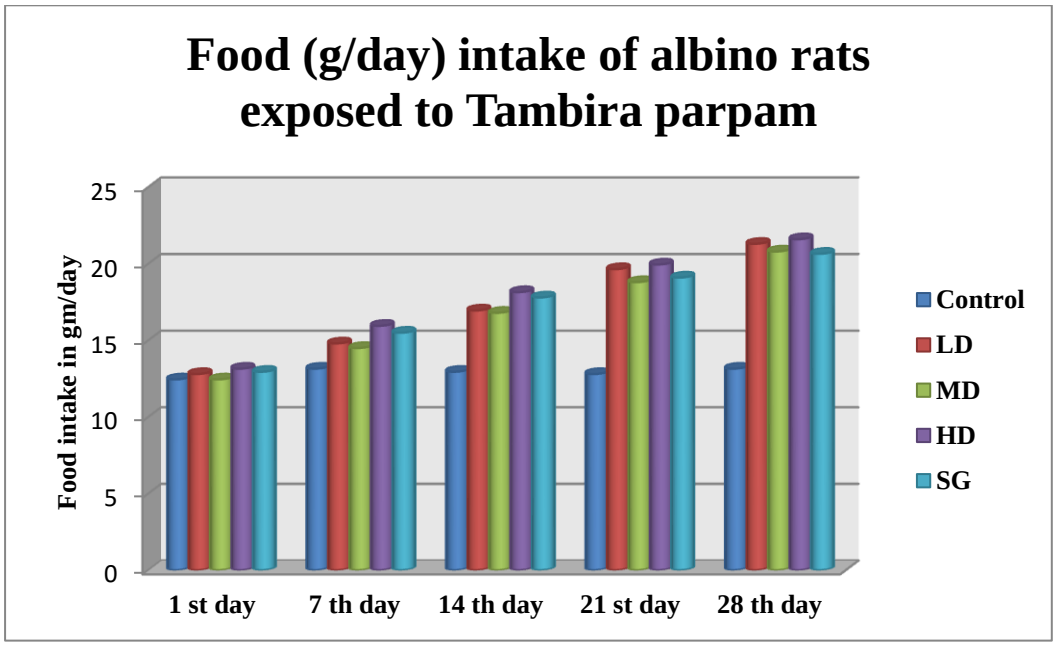
Food consumption of the animals significant difference in Food intake the test group animals were observed when compared with control group during the study period. (Table 8), but they are within physiological limit.

Table:8 Food (g/day) intake of albino rats exposed to Tambiraparpam

Dose (mg/kg/ day)	1st day	7 th day	14 th day	21st day	28 th day
Control	12.45±0.16	13.15±0.16	12.95±0.38	12.8±0.21	13.15±0.16
LD	12.8±0.21**	14.8±0.21**	16.95±0.38**	19.65±0.38**	21.3±0.32**
MD	12.45±0.16**	14.5±0.54**	16.8±0.54**	18.8±0.54**	19.95±0.71**
HD	13.15±0.16**	15.95±0.71**	18.15±1.25**	19.95±0.71**	21.65±0.71**
SG	12.98±0.38**	15.5±0.54**	17.8±0.54**	19.1±0.54**	20.65±0.71**

Values were expressed as mean± S.D. for N=6 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure:3



28 Day Repeated Dose Oral Toxicity Study

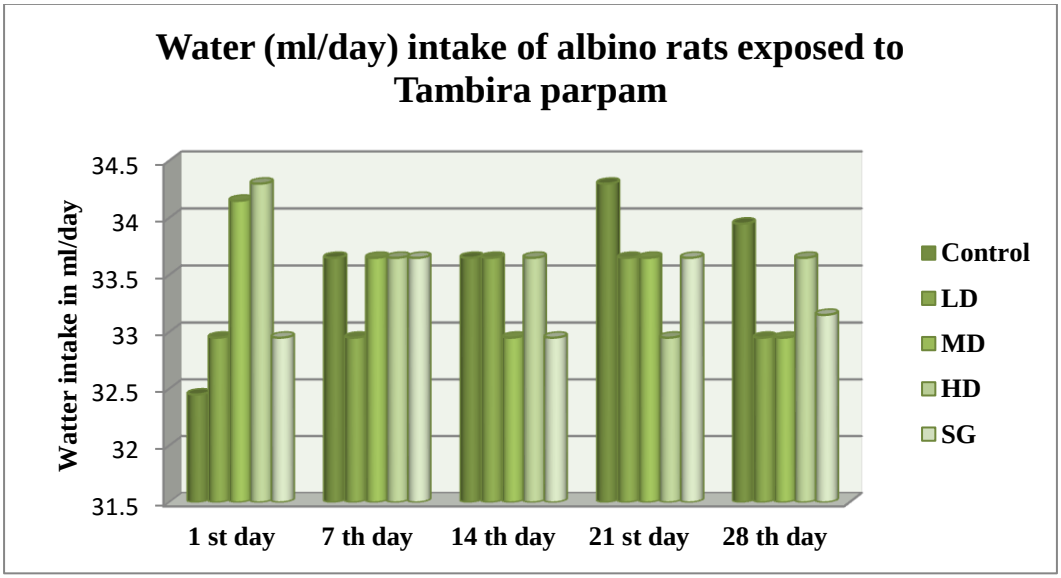
Water consumption the difference in Water intake of control and test group of animals observed during the study period. (Table 9)**,** There was significant difference occurs in the group low and mid at 28 days compared with control group.

Table:9 Water (ml/day) intake of albino rats exposed to Tambira parpam

Dose (mg/kg/	1 st day (ml/day)	7 th day (ml/day)	14 th day (ml/day)	21 st day (ml/day)	28 th day (ml/day)
Control	32.45±0.43	33.65±0.38	33.65±0.38	34.3±0.32	33.95±0.71
LD	32.95±0.38*	32.95±0.38*	33.65±0.38	33.65±0.38*	32.95±0.38**
MD	34.15±0.93*	33.65±0.38	32.95±0.38*	33.65±0.38*	32.95±0.38**
HD	34.3±1.86	33.65±0.38	33.65±0.38	32.95±0.38	32.95±0.38
SG	32.95±0.38	33.65±0.38	32.95±0.38*	33.65±0.38*	33.15±0.16*

Values were expressed as mean± S.D. for N=6 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure:4



28 Day Repeated Dose Oral Toxicity Study

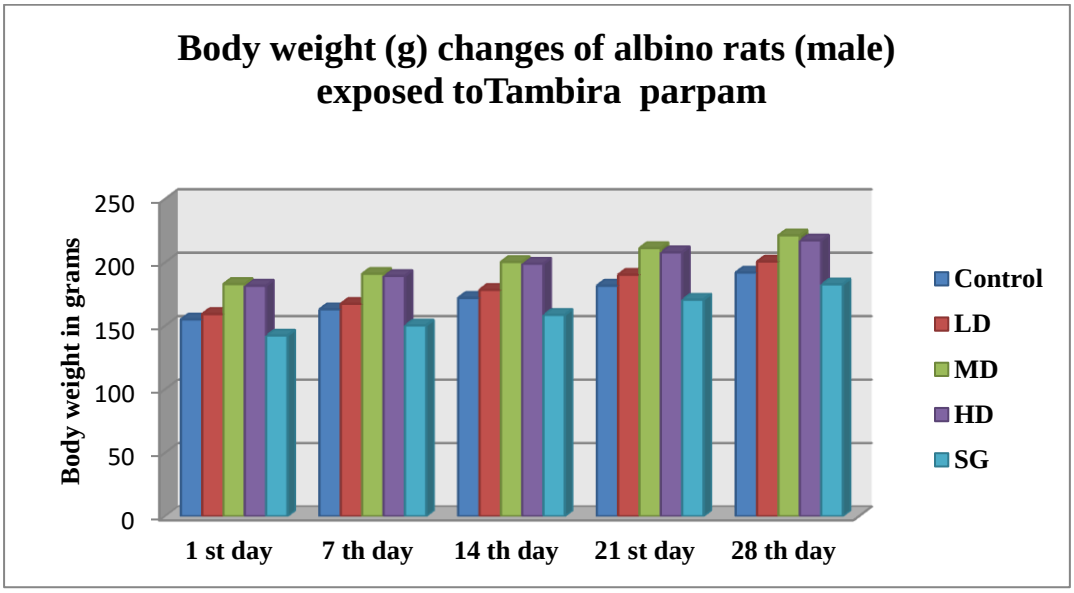
Body weight of both control and test dose group exhibited normal body weight throughout the study period. (Table 10)

Table: 10 Body weight (g) changes of albino rats (male) exposed toTambira parpam

Dose Mg/kg	1st day	7 th day	14 th day	21 st day	28 th day
Control	155±21.70	163±21.70	172±21.70	181.66±21.45	192±19.97
LD	159.3±21.07	167.33±21.07	178.33±20.5	190.33±18.71	200.66±18.14
MD	183±22.51	191±22.51	200.33±22.36	211.33±20.64	221.33±20.64
HD	181.66±8.50	189.33±8.02	199±7.21	208±5.29	217.33±5.50
SG	142.33±6.80	150.33±6.80	158.66±6.11	170.66±6.11	182.66±6.11

Values were expressed as mean± S.D. for N=6 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure:5



28 Day Repeated Dose Oral Toxicity Study

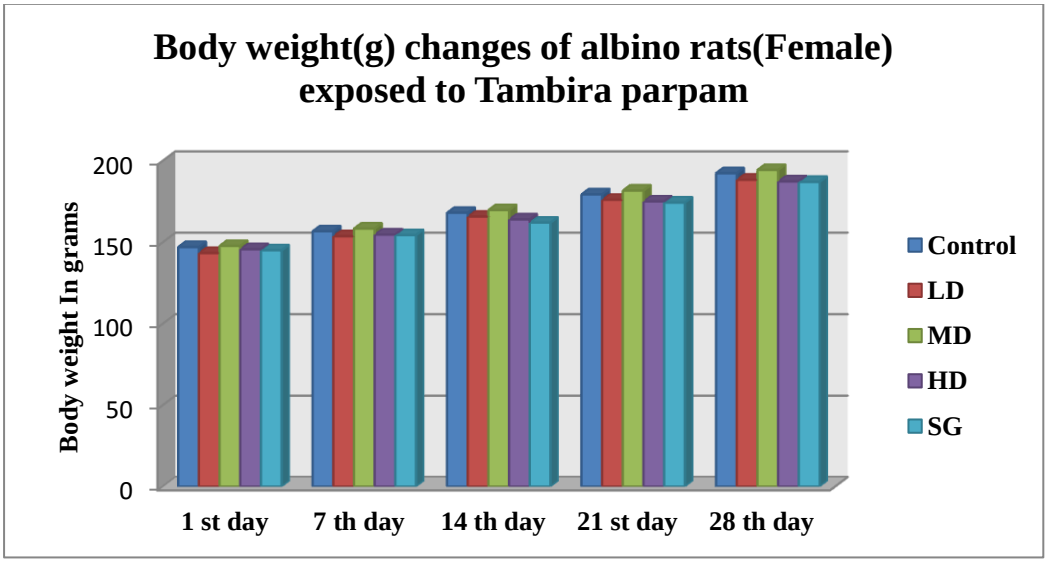
Body weight of both control and test dose group exhibited normal body weight throughout the study period. (Table 11)

Table:11 Body weight (g) changes of albino rats (female) exposed to Tambira parpam

Dose (mg/kg/ day)	1 st day	7 th day	14 th day	21 st day	28 th day
Control	147±4.35	156.66±4.16	168±4.35	179.33±8.08	192.33±3.78
LD	143.33± 7.24	153.66±7.09	165.66±7.09	176±6	180.33±6.02
MD	147.66±7.09	158.33±5.68	169.66±5.85	181.66±5.85	194.33±4.72
HD	145.66±5.85	154.66±6.80	164±6.55	175±6.55	187.33±6.11
SG	145± 7.93	154.33±7.09	162.66±8.08	174.33±8.02	187±7.54

Values were expressed as mean± S.D. for N=6 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure:6



28 Day Repeated Dose Oral Toxicity Study

The results of the **Haematological investigations** conducted at the end of the study, the groups revealed slightly significant changes in levels of haematological parameters, when compared with control group, and post retrieval group haematological parameters towards normal, when compared with control group. (Table 12)

Table: 12 Effect of Tambira parpam on Haematological Parameters

Parameter	Control	LD	MD	HD	SG
RBC ($\times10^6$µl)	4.08±0.09	5.75±1.57	5.7±1.67	6.06±1.16*	6.28±0.64*
WBC ($\times10^3$µl)	8.93±0.48	13.31±8.20	10.65±3.15	12.98±3.13	10.8±2.10
PLT ($\times10^3$µl)	792.8±93.33	560±176.41*	771.6±127.12	641.6±148.2	743±101.30
HGB (g/dl)	12.5±0.74	13.81±1.28	11.81±2.42	12.8±1.54	11.66±2.32
Neutrophils 10^3/mm^3	2.01±0.47	2.05±0.55	2.1±0.50	14.61±28.6	2.45±0.79
Lymphocyte (%)	76.4±1.52	75.56±10.59	73.01±6.84	83.58±5.60	77.41±4.33
Monocyte (%)	3.18±0.11	2.41±0.82	14.35±28.8	3.51±1.19	2.51±0.90
eosinophil's (%)	1.3±0.15	1.23±0.20	1.41±0.28	1.45±0.21	1.55±0.23
Basophils (%)	0.66±0.51	0.33±0.51	0.5±0.54	0.33±0.51	0.16±0.40
MCH (pg)	20.95±1.0	18.25±1.42	18.5±2.6	17.85±1.99*	16.41±2.55**
MCV (fl)	62.06±2.65	59.3±3.74	56.4±8.76	57.53±7.7	53.68±6.7

Values were expressed as mean± S.D. for N=6 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure: 7

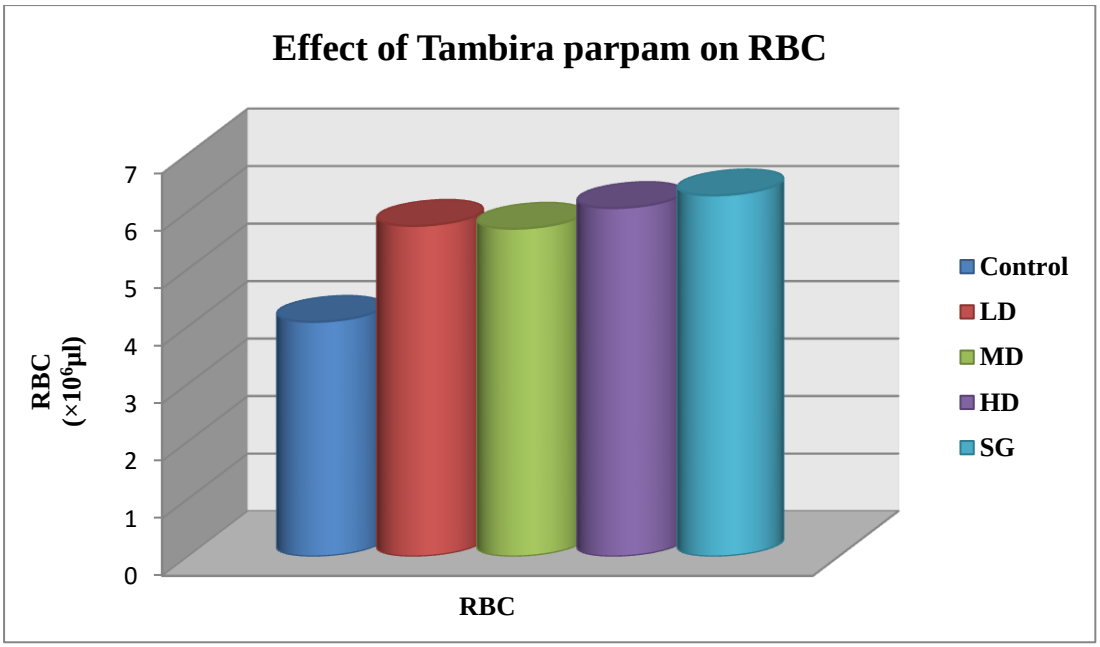


Figure: 8

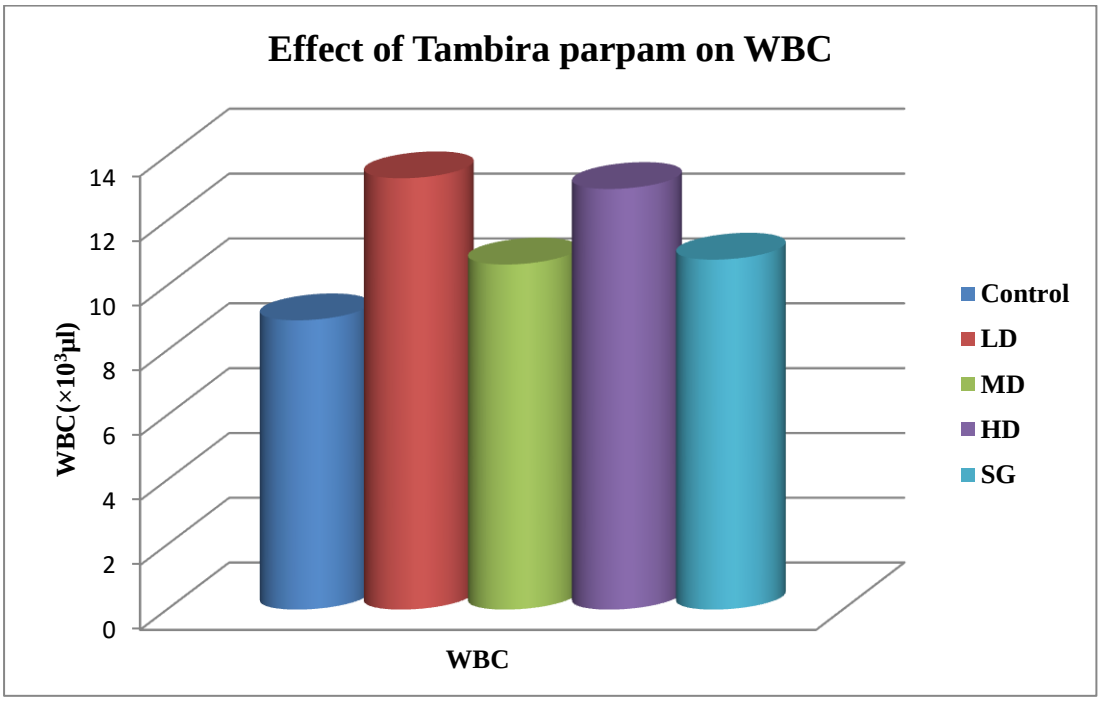


Figure:9

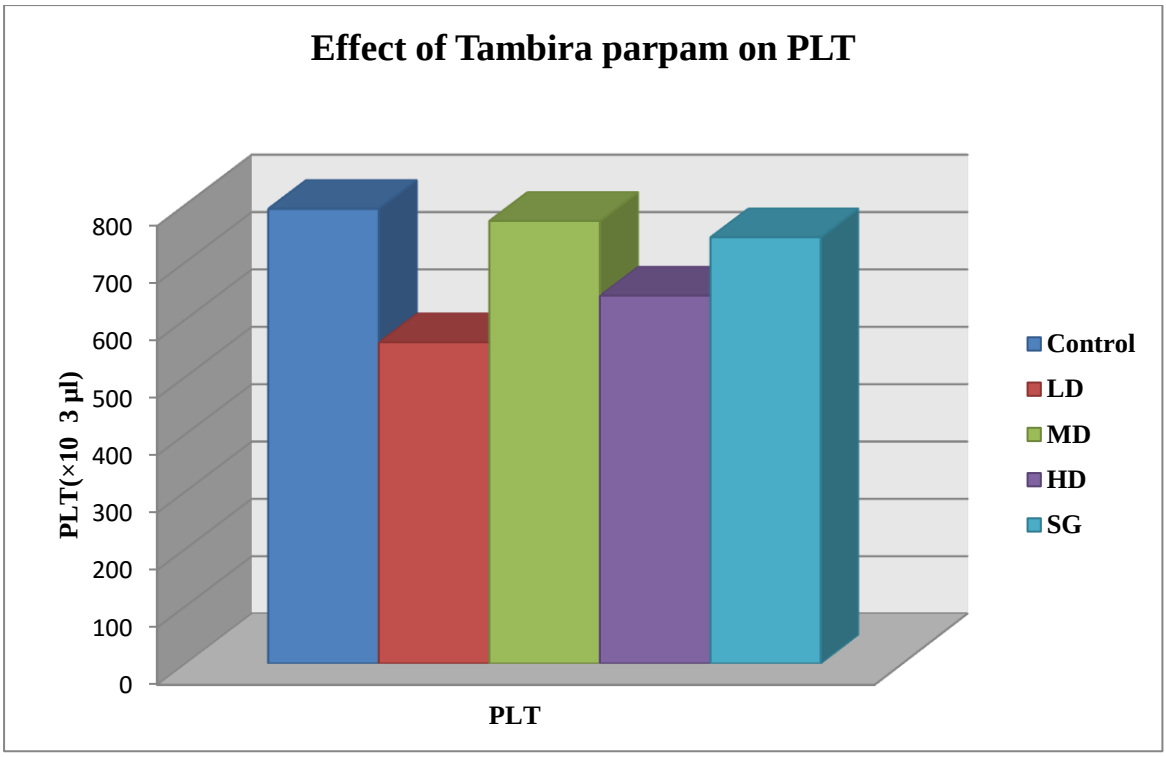


Figure: 10

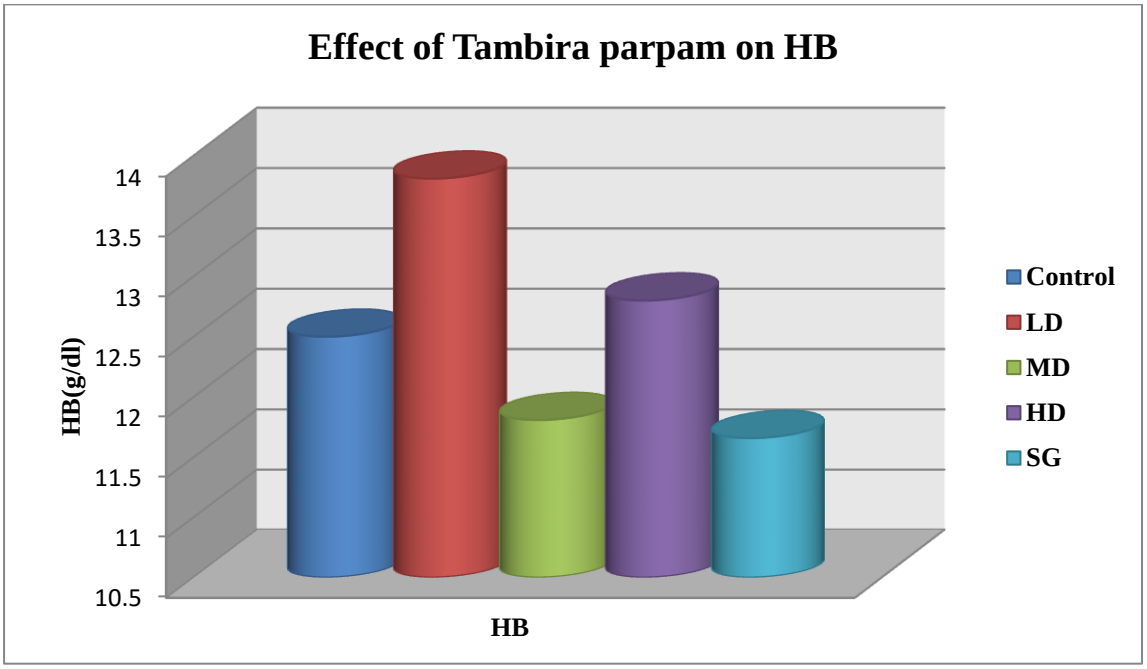
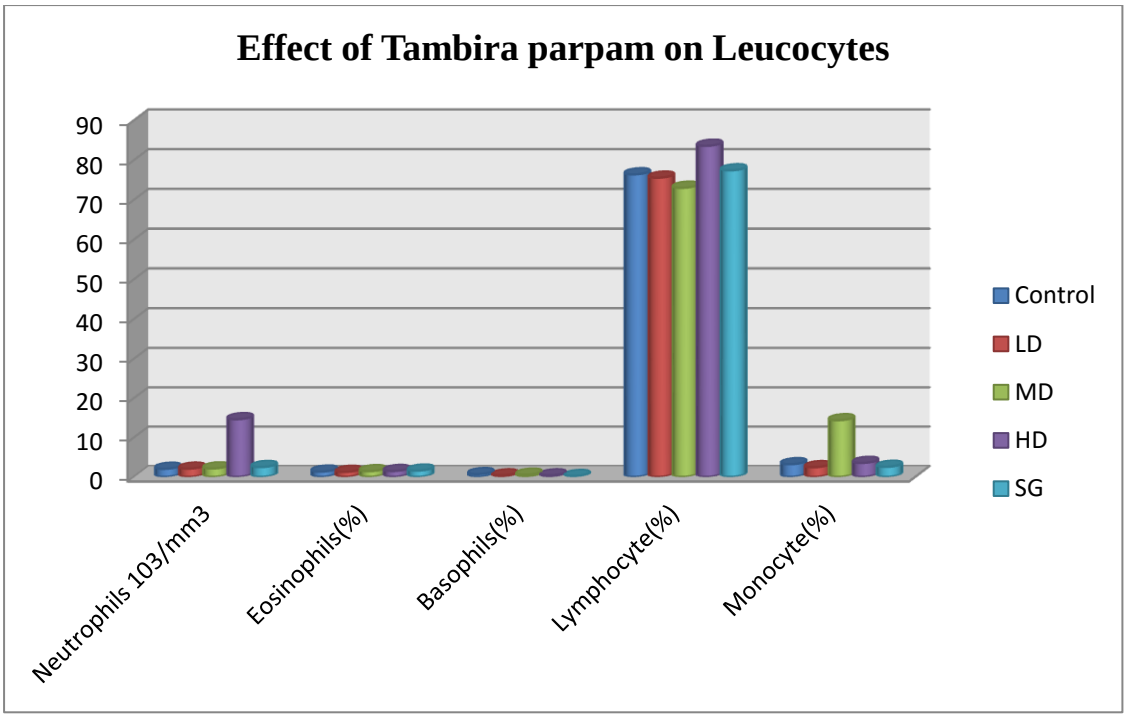


Figure: 11



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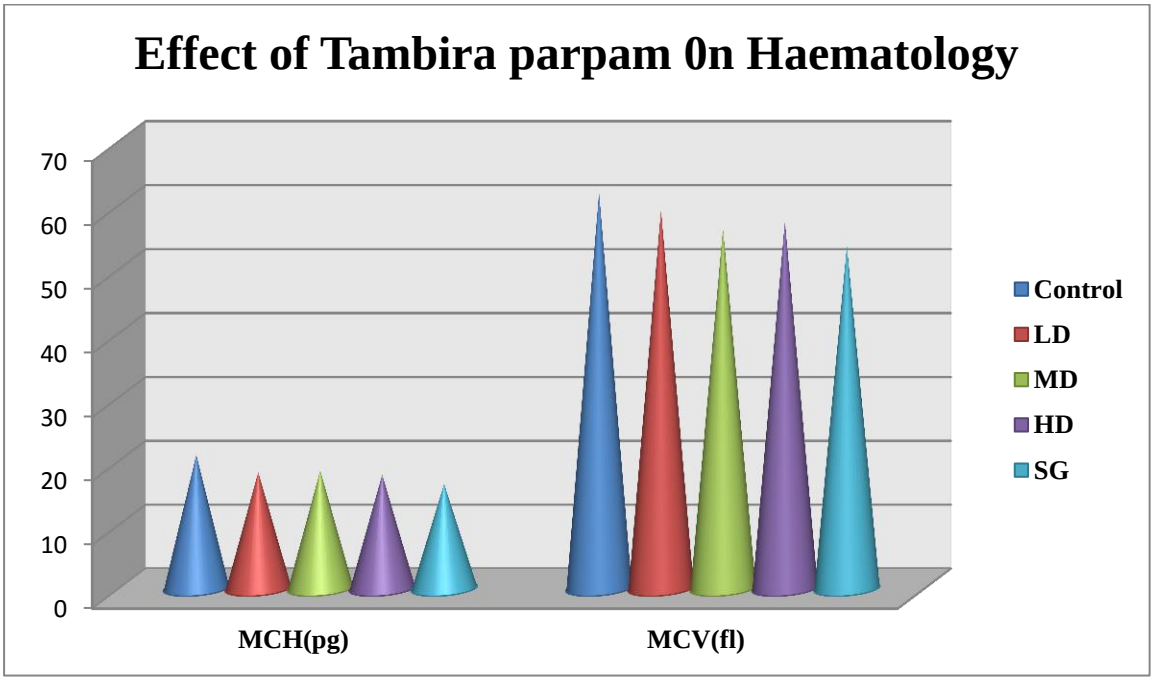


28 Day Repeated Dose Oral Toxicity Study

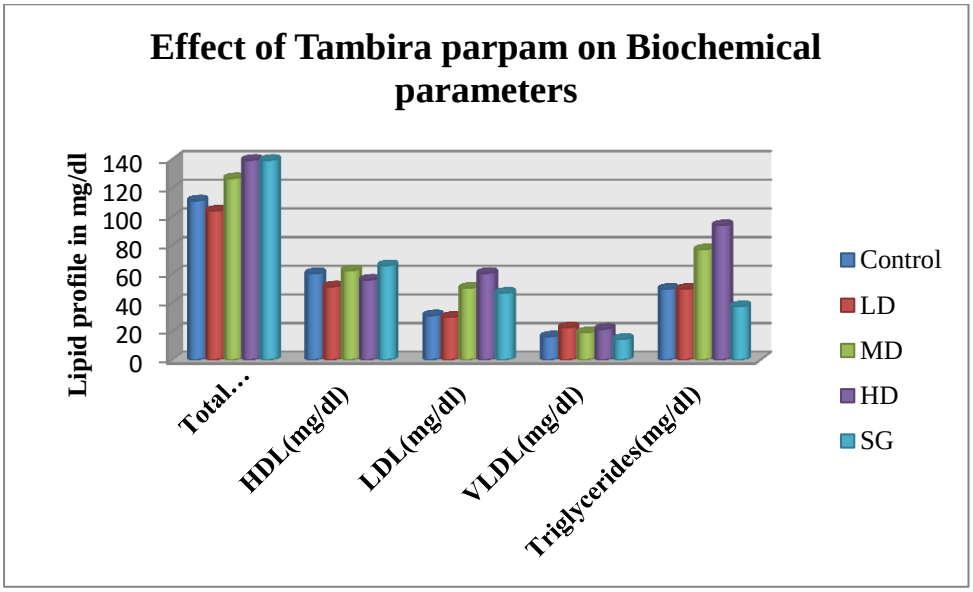
Biochemical investigations were conducted at the end of the study and the results were recorded. In test groups there was significant changes present in biochemical parameters, when compared with the control group. At the values were normal biological limits. (Table 13)

Table:13 Effect of Tambira parpam on Biochemical parameters

Dose (mg/kg)	Control	LD	MD	HD	SG
Total cholesterol (mg/dl)	111.26±1.16	104.1±9.90	126.8±25.0	139.51±8.65**	127.46±11.1
HDL (mg/dl)	60.5±4.08	51.33±7.03	62.5±7.28	56±6.6	66±6.35
LDL (mg/dl)	31.16±5.03	30±9.81	50.3±22.9*	60.66±7.52**	46.83±6.64
VLDL (mg/dl)	16.43±2.72	22.7±4.39	19.28±8.23	21.71±3.52	14.63±1.89
Triglycerides (mg/dl)	49.66±2.33	49.6±16.30	77.16±22.6	94±27.5**	37.5±12.5

Values were expressed as mean± S.D. for N=6 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure:13



28 Day Repeated Dose Oral Toxicity Study

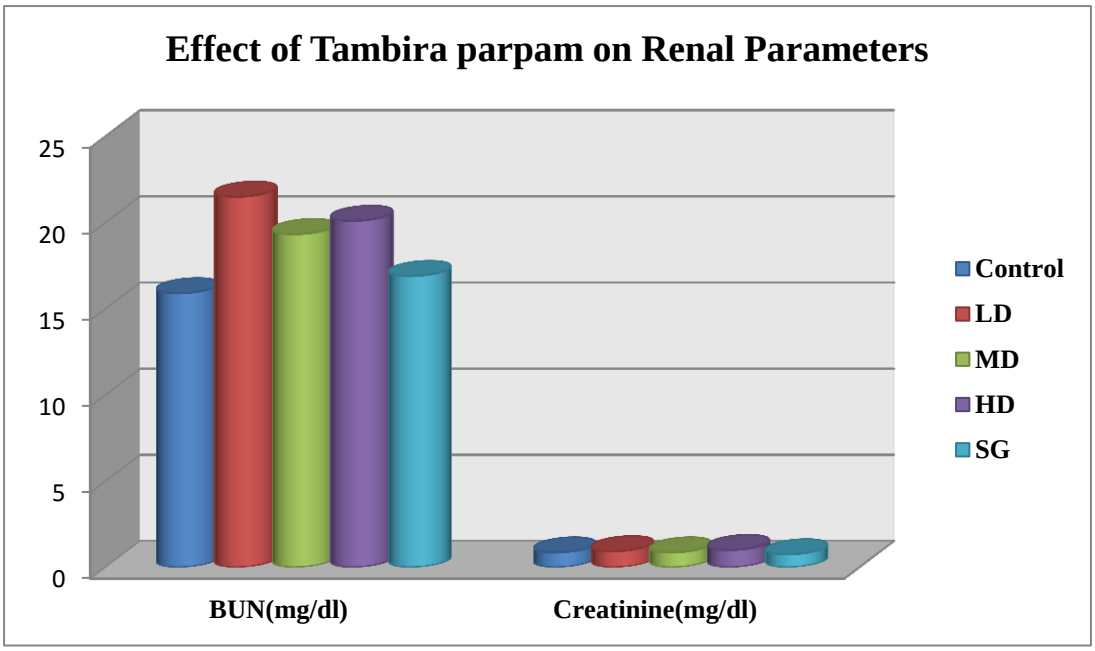
The results of the renal functions test conducted at the end of study, test groups revealed significant changes in levels of renal parameters, when compared with control group, and post retrieval group Renal function parameters towards normal, when compared with control group.(Table 14)

Table:14 Effect of Tambira parpam on Renal Parameters

Dose(mg/kg)	Control	LD	MD	HD	SG
BUN(mg/dl)	15.91±2.2	21.5±2.8**	19.33±2.94	20.1±2.48*	16.9±3.33
Creatinine (mg/dl)	0.86±0.12	0.93±0.12	0.86±0.24	1±0.17	0.75±0.22

Values were expressed as mean± S.D. for N=6 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure:14



28 Day Repeated Dose Oral Toxicity Study

The results of the liver function test conducted at the end of the study, test groups revealed significant changes in levels of liver parameters, when compared with control group, and post retrieval group Liver function parameters towards normal, when compared with control group

Table:15 Effect of Tambira parpam on Hepatic ParameterS

Dose(mg/kg)	Control	LD	MD	HD	SG
Total Bilirubin(mg/dl)	0.48±0.15	0.66±0.16	0.58±0.21	0.8±0.16*	0.45±0.2
SGOT(U/L)	101±14.8	96.6±49.1	134.6±32	143.6±12.0	122±27.1
SGPT(U/L)	29.8±2.7	55±7.5**	53±7.1**	52.5±6.9**	36±10.6

Values were expressed as mean± S.D. for N=6 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure:15

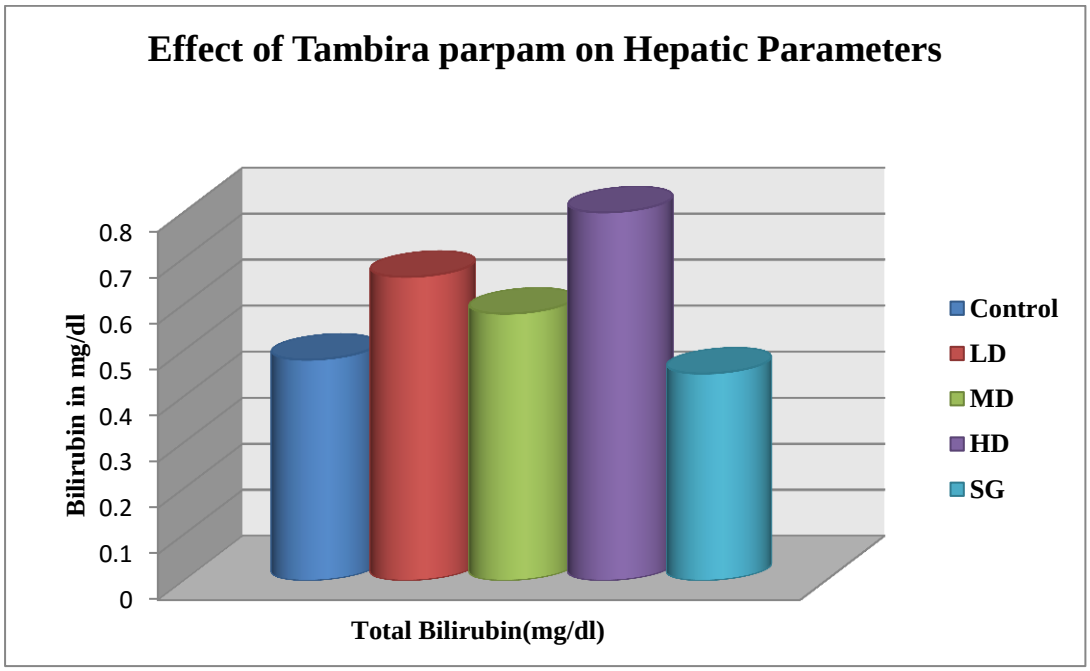
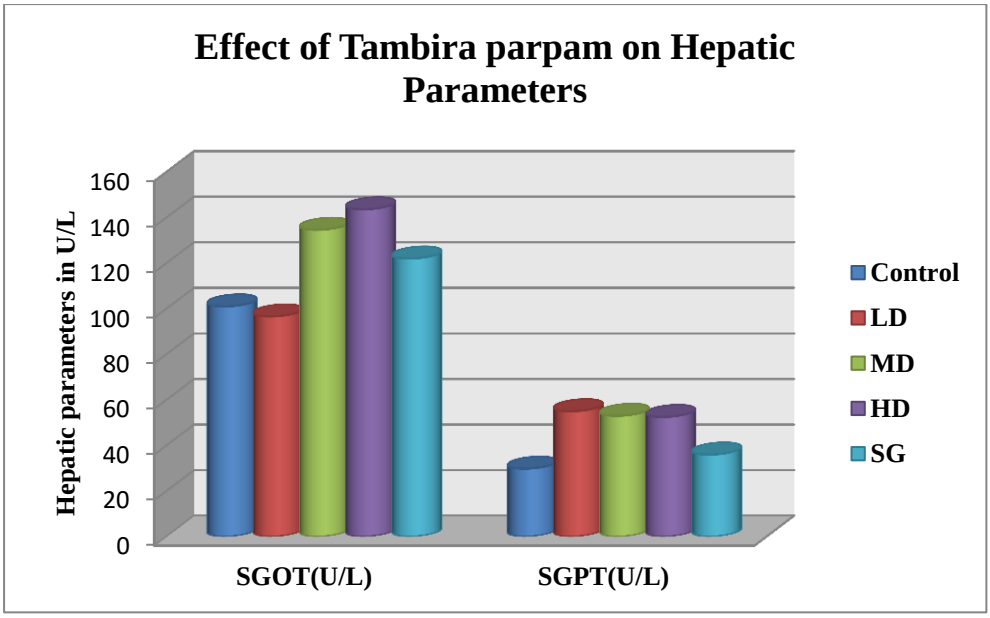


Figure:16


Effect of Tambira parpam on Hepatic Parameters
Hepatic parameters in U/L
160
140
120
100
80
60
40
20
0
SGOT(U/L)
SGPT(U/L)
Control
LD
MD
HD
SG

28 Day Repeated Dose Oral Toxicity Study

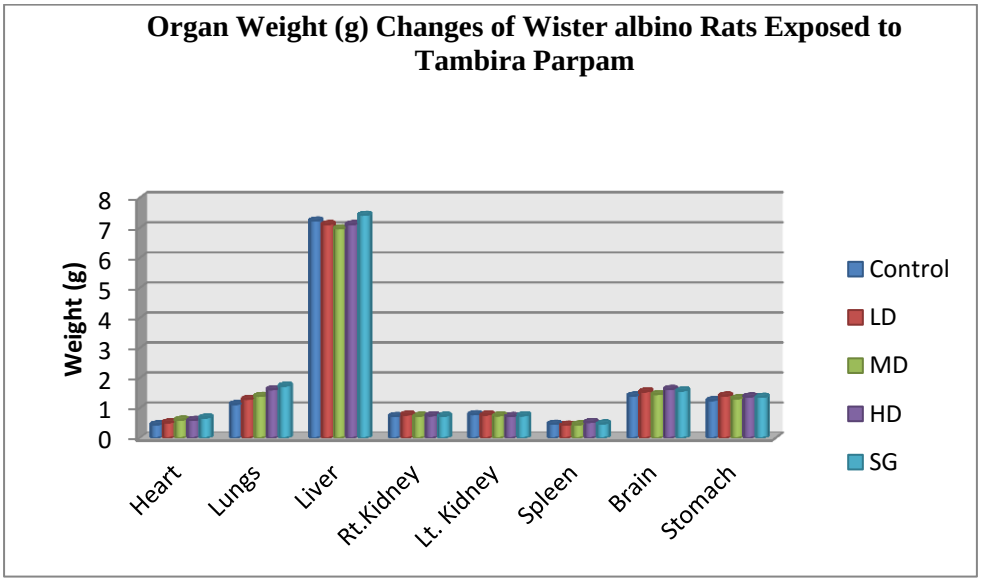
The Organ weight no difference in organ weight of control and test group observed after 28th days repeated oral toxicity study period, and satellite group was sacrificed after 14 days of drug withdrawal.(Table 16)

Table: 16 Organ Weight (g) Changes of Wister albino Rats Exposed to Tambira Parpam

Organs	Control	Low dose	Mid dose	High dose	Satellite group
Heart	0.45±0.02	0.52±0.10	0.61±0.11	0.60±0.01	0.68±0.13
Lungs	1.13±0.21	1.3±0.34	1.4±0.02	1.62±0.51	1.74±0.57
Liver	7.24±1.77	7.12±1.12	6.98±0.11	7.12±1.65	7.43±1.13
Rt. Kidney	0.73±0.07	0.78±0.41	0.74±0.43	0.75±0.20	0.74±0.13
Lt. Kidney	0.79±0.09	0.78±0.07	0.75±0.01	0.73±0.19	0.75±0.15
Spleen	0.47±0.21	0.44±0.08	0.45±0.02	0.53±0.13	0.48±0.10
Brain	1.41±0.12	1.55±0.08	1.46±0.02	1.63±0.09	1.58±0.11
Stomach	1.26±0.16	1.41±0.09	1.32±0.16	1.38±0.18	1.37±0.21

Values were expressed as mean± S.D. for N=6 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure:17



28 Day Repeated Dose Oral Toxicity Study

Histopathology of Heart

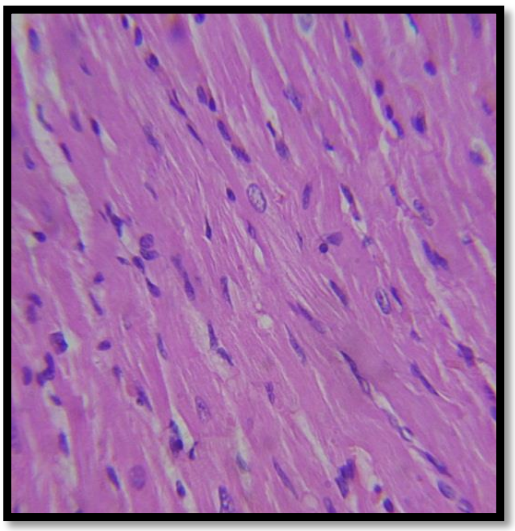


Plate a. Control

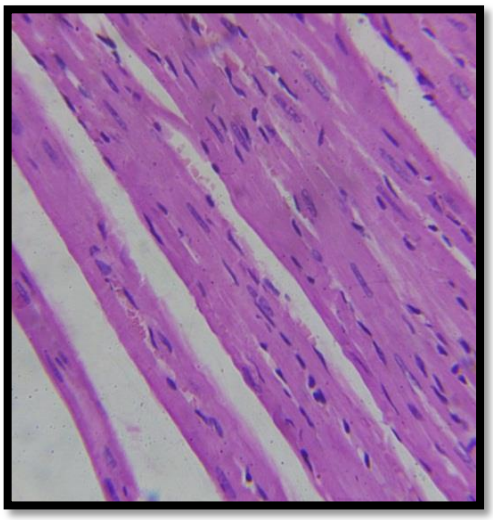


Plate b. Low dose group

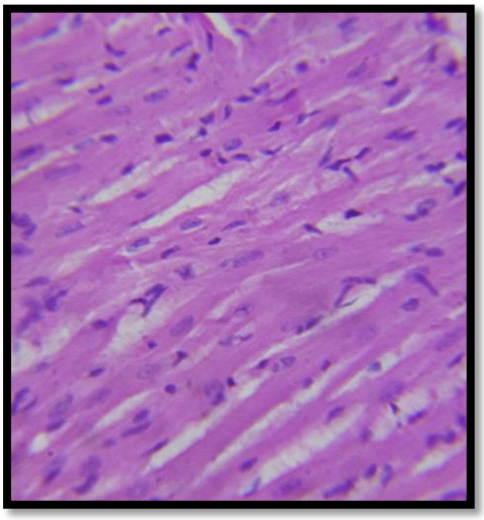


Plate c. Mid dose group

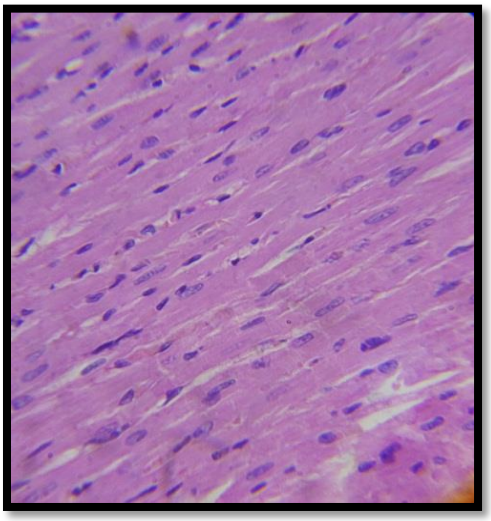


Plate d. High dose group

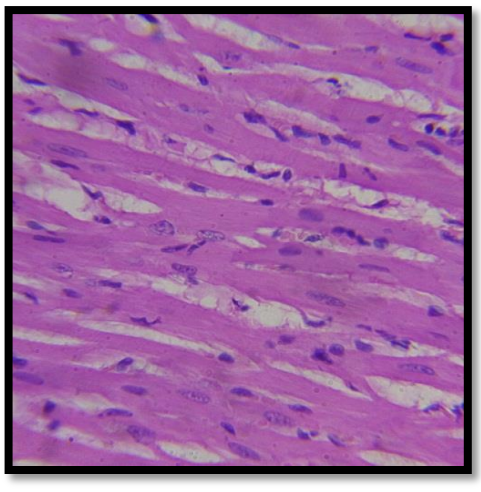


Plate e. Satellite group

Plate a

- Nucleus appears prominent with a regular arrangement of fibers. No evidence of pyknotic nucleus.
- No evidence of collagen deposition in the myocardium.

Plate b

- Normal network of myocardial fibres was observed
- No evidence of atherosclerosis and thrombosis

Plate c

- Nuclei of cardiomyocytes appears regular size and shape
- Arrangement of cardiac myofibers was normal

Plate d

- Cardiac fibers appears normal with regular striations

Plate e

- The appearance of cardiomyocyte was normal with the dark nuclear region. The nuclei of muscle fibers appear central arrangement.
- Myocardial tissue appears normal with orderly striated heart muscle fibers and a clear nuclear and muscle bands.

28 Day Repeated Dose Oral Toxicity Study

Histopathology of Lungs

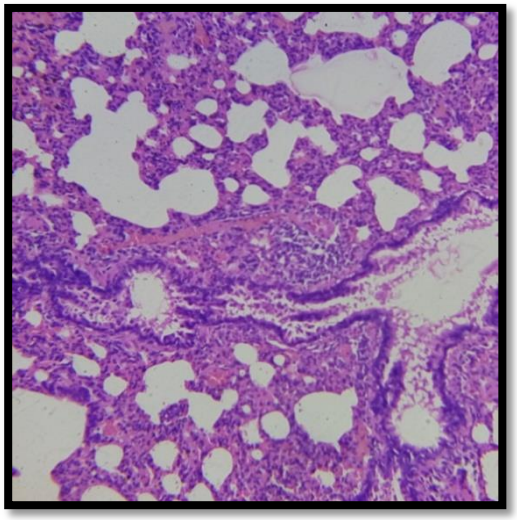


Plate a. Control

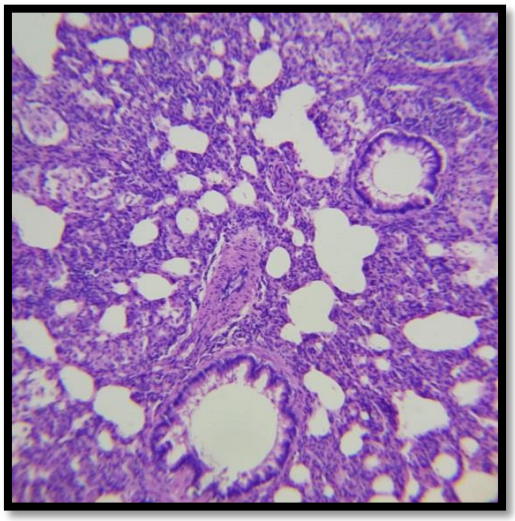


Plate b. Low dose group

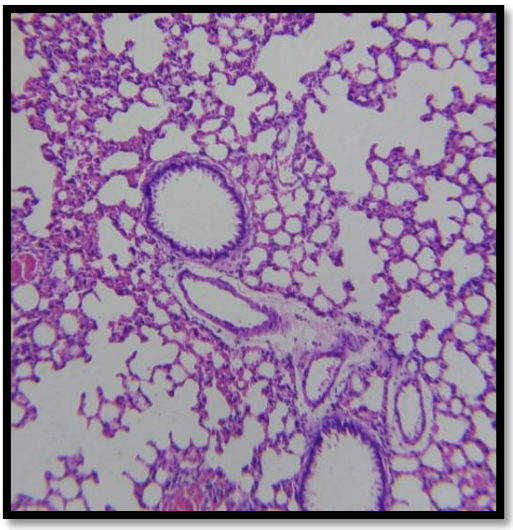


Plate c. Mid dose group

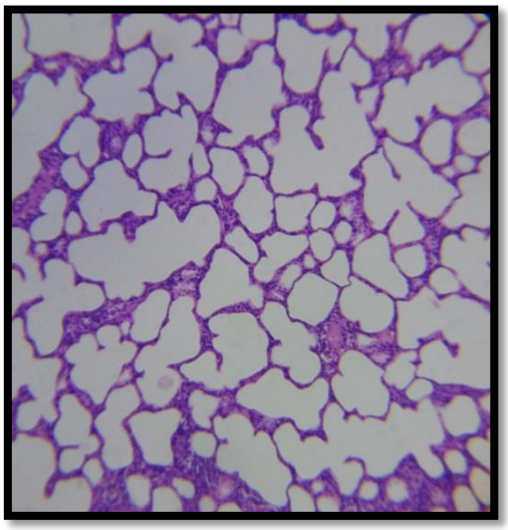


Plate d. High dose group

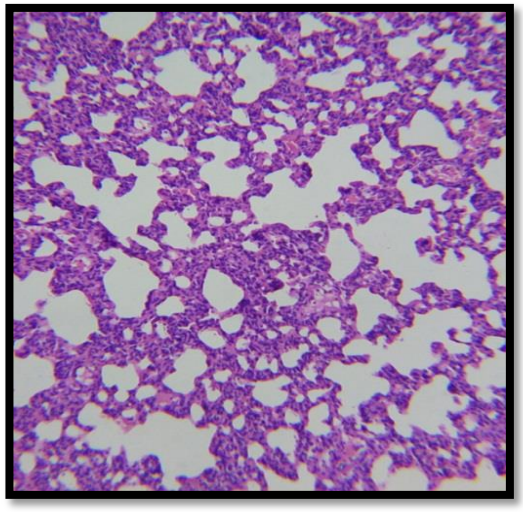


Plate e. Satellite group

Plate a

- Lung parenchyma appears normal with regular arrangement of alveoli and alveolar sac with no signs of lymphocyte infiltration and pulmonary fibrosis
- No evidence of lymphocyte aggregation in deep airway layers

Plate b

- Normal lung parenchyma with regular airway histology was observed
- No evidence of perivascular cuffing

Plate c

- No evidence of lymphocyte proliferation
- Appearance of vascular sheath and perivascular regions are normal

Plate d

- No signs of airway secretion and bronchial secretion
- Bronchial blood vessels and connective tissue appears normal with no signs of pulmonary oedema

Plate e

- Perivascular region appears normal, Alveolar septa and wall appeared to widen and normal
- Lung parenchyma appears normal with regular arrangement of alveoli and alveolar sac with no signs of lymphocyte infiltration and pulmonary fibrosis.

28 Day Repeated Dose Oral Toxicity Study

Histopathology of Liver

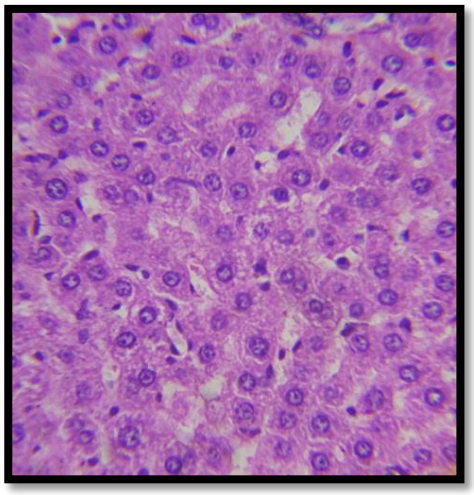


Plate a. Control

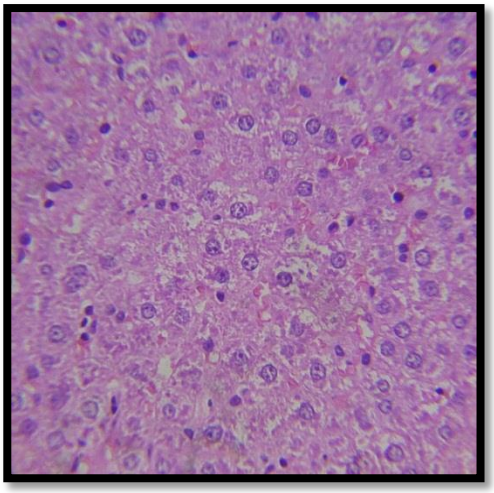


Plate b. Low dose group

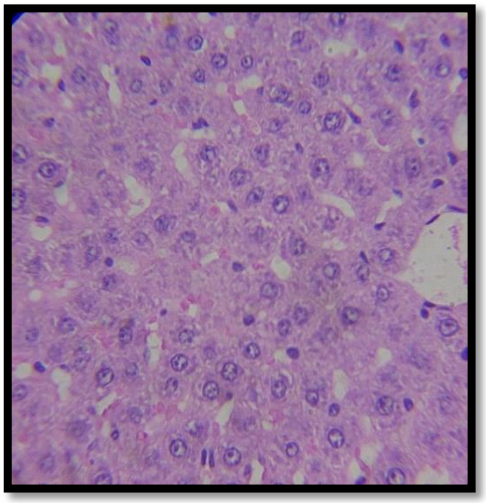


Plate c. Mid dose group

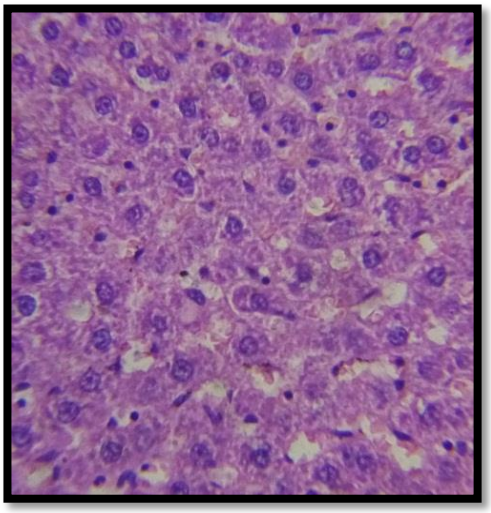


Plate d. High dose group

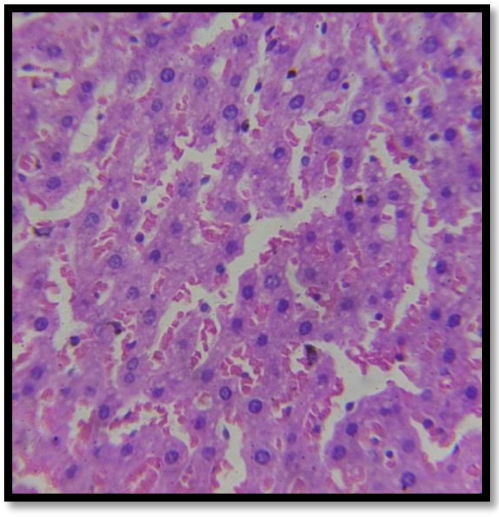


Plate e. satellite group

Plate a

- Hepatocytic cytoplasm appears normal. The centrilobular hepatocytes appears normal with stained cytoplasm
- No evidence of mesenchymal reaction on to the hepatic parenchyma.

Plate b

- Numerous hepatocytes appears with shrunken nucleus
- No signs of nodular degeneration and cirrhosis.
- No evidence of collagen (fibrosis)

Plate c

- Increased numbers of Kupfer cells were observed
- Occasional migration of inflammatory cells

Plate d

- Diffused vascular changes were observed in the mid-zonal region
- Hepatocytes appear variably pale with mild congestion on central vein
- Mild discrete cytoplasmic vacuoles and rare foamy cytoplasm were observed

Plate e

- Extensive periportal degenerative change was observed
- Hepatocytes rarely projects dark pyknotic shrunken nuclei

28 Day Repeated Dose Oral Toxicity Study

Histopathology of Stomach

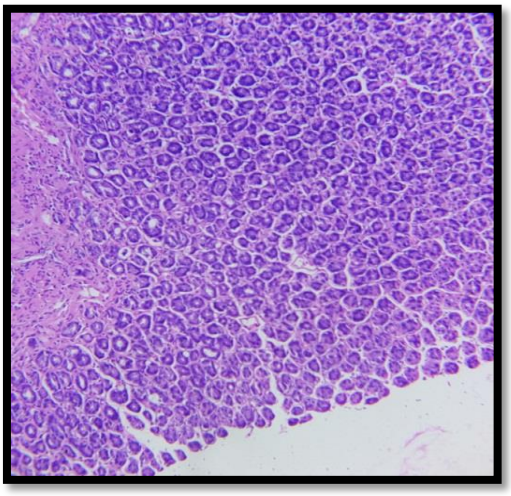


Plate a. Control

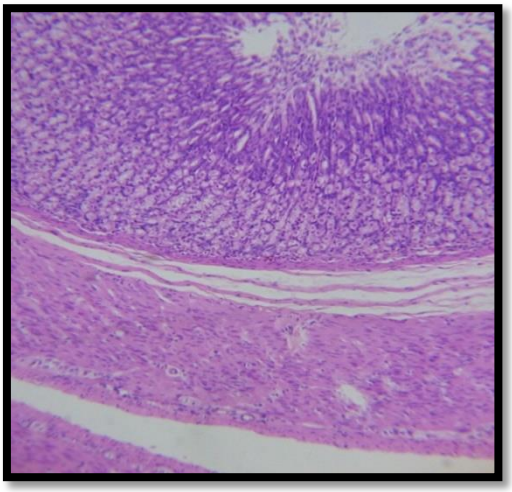


Plate b. Low dose group

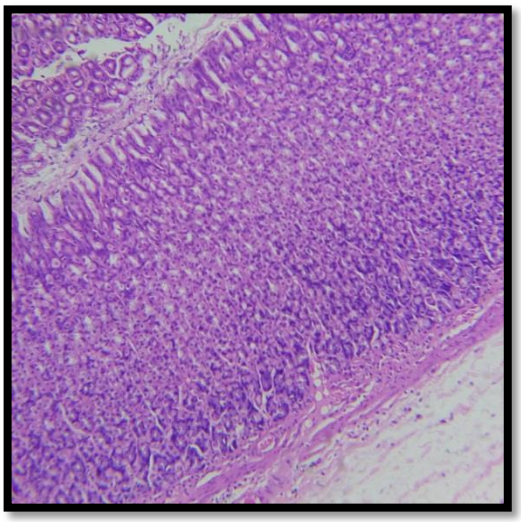


Plate c. Mid dose group

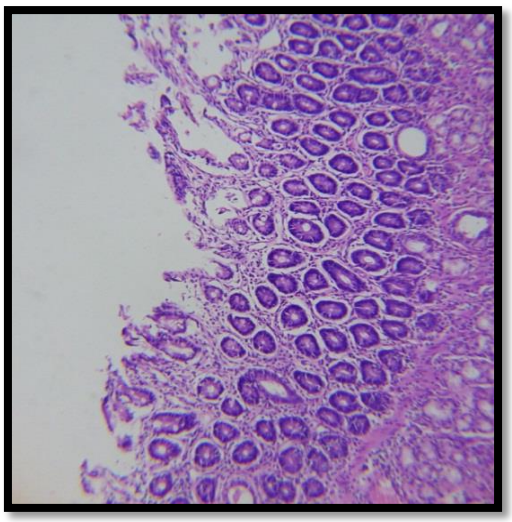


Plate d. High dose group

Plate e. Satellite group

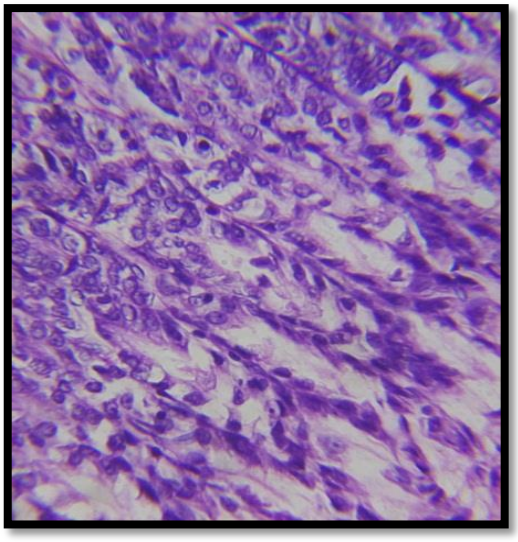


Plate a

- Mucosal wall appears normal with regular arrangement of connective tissue
- Histology of gastric wall composed of normal mucosa, muscular mucosa, sub mucosa, muscularis propiria and adventitia

Plate b

- Intracytoplasmic zone of mucosa appears normal
- Histology of gastric wall composed of normal mucosa, muscular mucosa, sub mucosa, muscularis propiria, and adventitia.

Plate c

- Light microscopic observation stomach reveals normal histology of gastric wall composed of normal mucosa, muscular mucosa, sub mucosa, muscularis propiria, and adventitia. No signs of ulceration were observed

Plate d

- The appearance of the glandular lumen was normal. Lamina propria appears normal with no evidence of infiltration and inflammation.

Plate e

- Light microscopic observation of both the sample reveals normal histology of rat gastric wall composed of mucosa, muscular mucosa, sub mucosa, muscularis propiria, and adventitia

28 Day Repeated Dose Oral Toxicity Study

Histopathology of Spleen

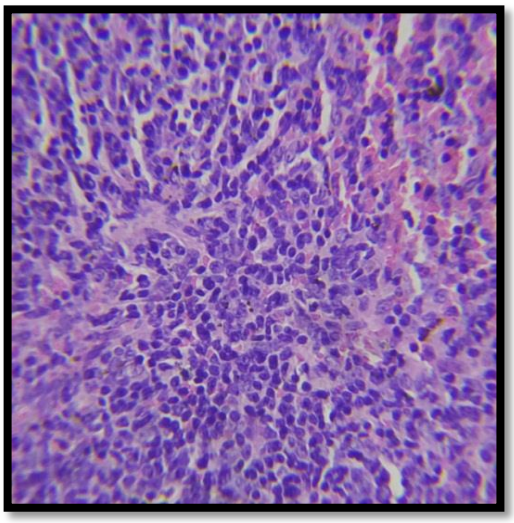


Plate a. Control

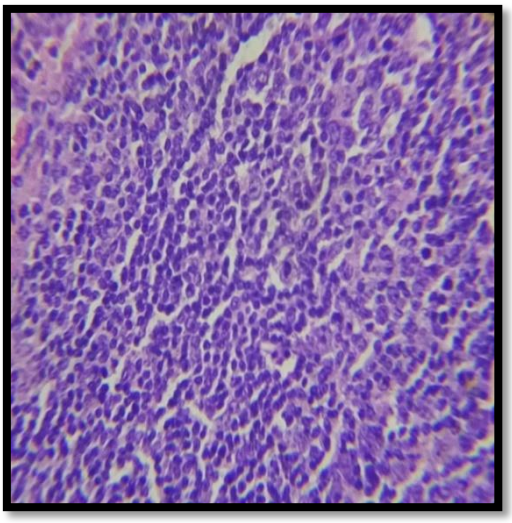


Plate b. Low dose group

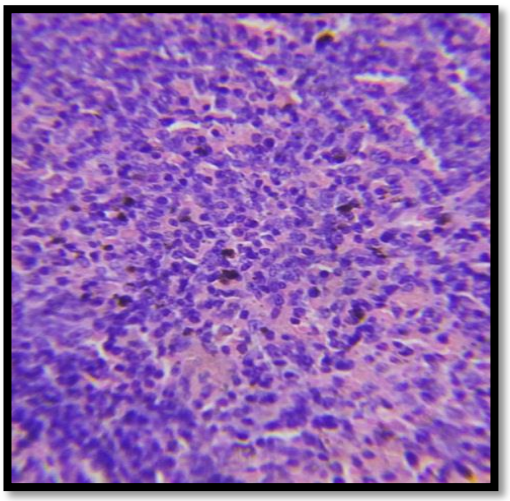


Plate c. Mid dose group

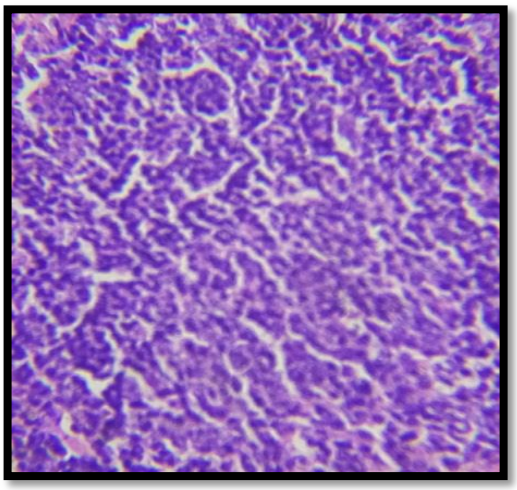


Plate d. High dose group

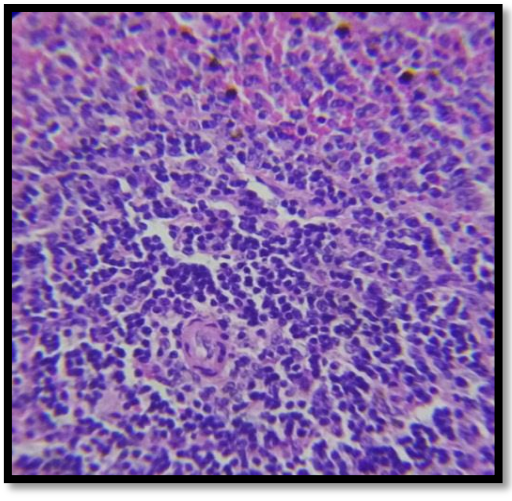


Plate e. Satellite group

Plate a

- The appearance of red pulp and marginal sinus are normal. No abnormalities found in lymph nodes

Plate b

- Appearance of marginal sinus was normal
- Lymphoid follicles appear normal. Marginal sinus (MS) of the rat and its sinus lining cells appear normal. Erythropoietic cells (EP) are scattered throughout the red pulp.

Plate c

- Germinal centre, Follicle and Central artery appears normal

Plate d

- Mild reduction in cellularity and size of the red pulp

Plate e

- Erythropoietic cells (EP) are scattered throughout the red pulp. No abnormalities found in the lymph node.

28 Day Repeated Dose Oral Toxicity Study

Histopathology of Kidney

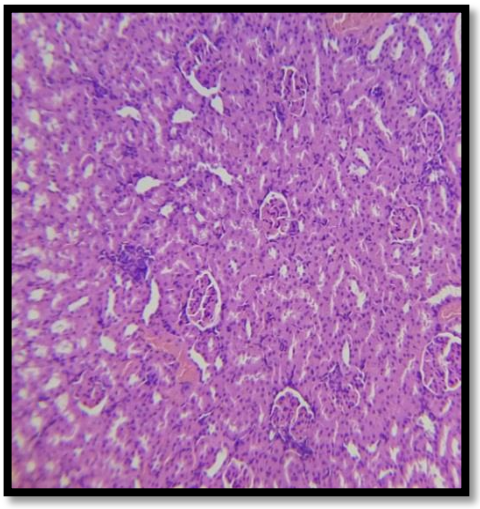


Plate a. Control

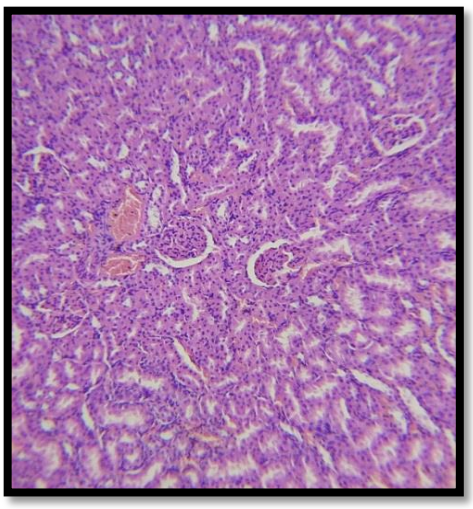


Plate b. Low dose group

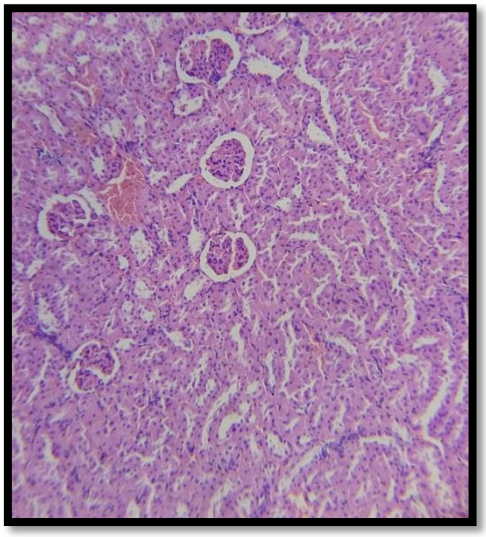


Plate c. Mid dose group

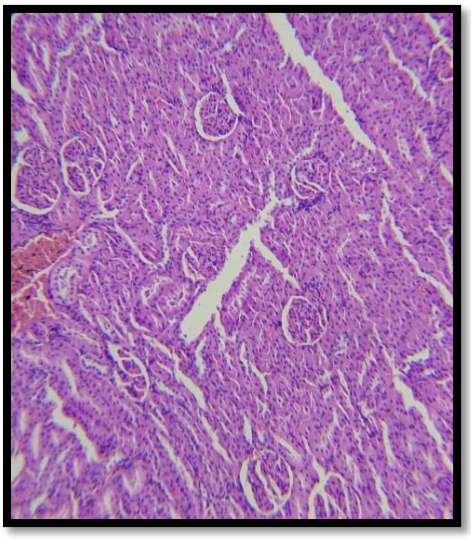


Plate d. High dose group

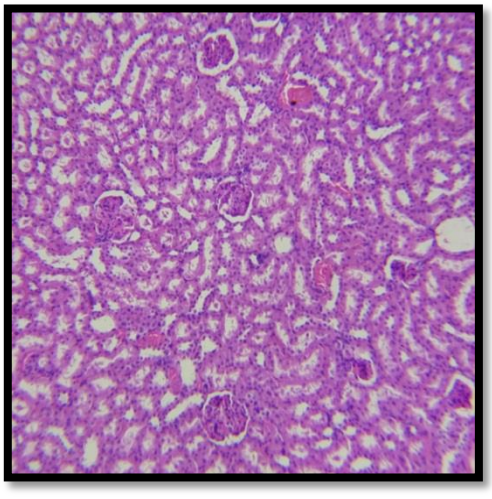


Plate e. satellite group

Plate a

- Glomerulus (G) surrounded by a narrow capsular space and the parietal layer of Bowman's capsule.
- Note the proximal convoluted tubules (P) and the distal convoluted tubules (D)
- Epithelial lining on proximal convoluted tubule appears normal
- The lumen of distal convolutes tubule and collecting duct was normal.

Plate b

- Presence of tubular cast was evident with widening proximal and distal convoluted tubule
- Shrunken glomeruli with wide bowman’s space.

Plate c

- Swollen tubular basement membrane
- The lining epithelial cells of the renal tubules shown pyknosis of the nuclei

Plate d

- Section showed shrunken glomeruli (G) with widening capsular Bowman's space
- Rare appearance of intercapillary sclerosis was observed

Plate e

- Glomerular degeneration with mild derangement in mesenchymal density
- Alteration in thickness of proximal convoluted tubule
- Mild tubular degeneration with increase bowman’s space
- Renal tubule with the mild swollen epithelial cell.

28 Day Repeated Dose Oral Toxicity Study

Histopathology of Testis

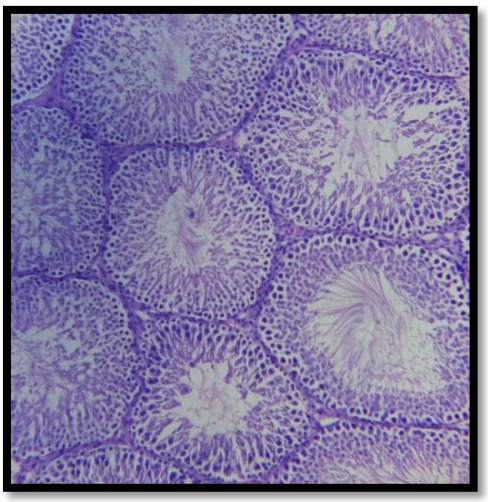


Plate a. Control

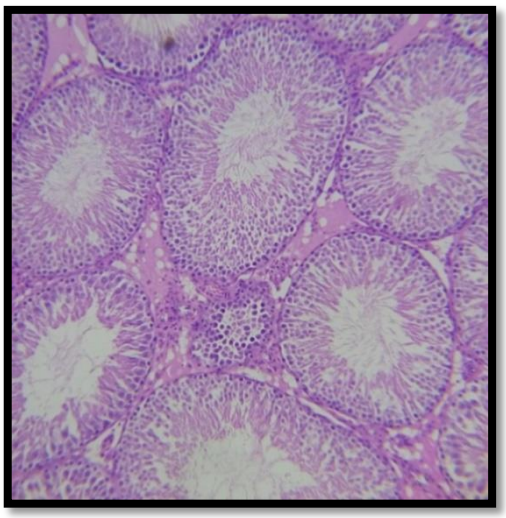


Plate b. Low dose group

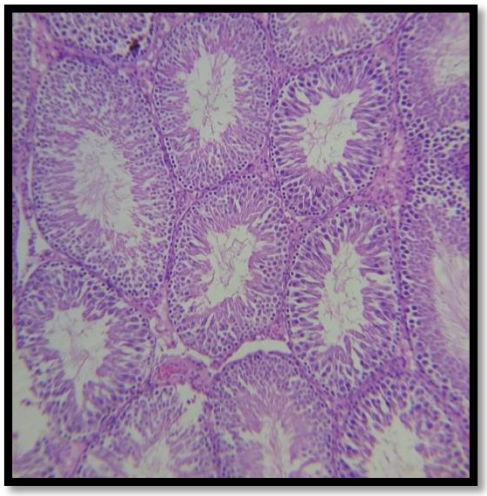


Plate c. Mid dose group

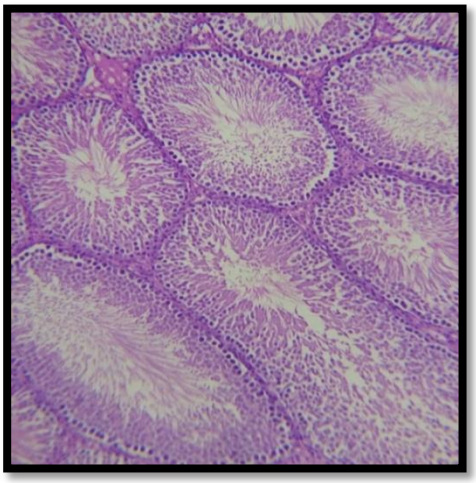


Plate d. High dose group

Plate e. Satellite group

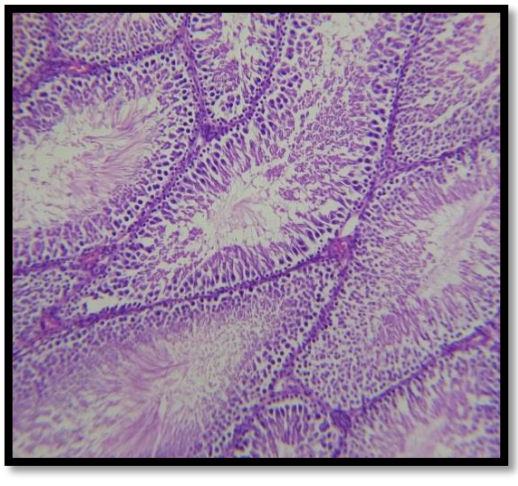


Plate a

- The presence of mature somatic cells projects the perfect histomorphology of testicular cells were observed.
- Primary spermatocytes with a largely centred nucleus and dense chromatin were observed.

Plate b

- Normal Sertoli cell aligned properly on the basement membrane with oval dome shaped nucleus shows the normal morphology of the seminiferous tubule was observed.

Plate c

- Histo cytology of testicular tissue shows well-differentiated germ cells with respect of spermatogonia includes spermatid and sperm were observed.

Plate d

- The appearance of Leydig cells, interstitial tissue, seminiferous tubule, Sertoli cells, and spermatogonia were normal

Plate e

- The Presence of mature somatic cells projects the perfect histomorphology of testicular cells were observed. Primary spermatocytes with a large centred nucleus and dense chromatin were observed.

28 Day Repeated Dose Oral Toxicity Study

Histopathology of Uterus

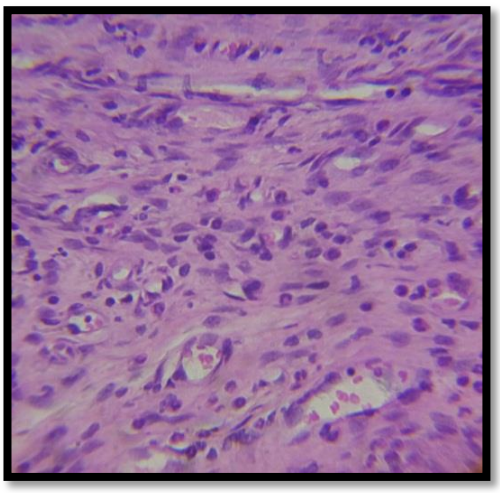


Plate a. Control

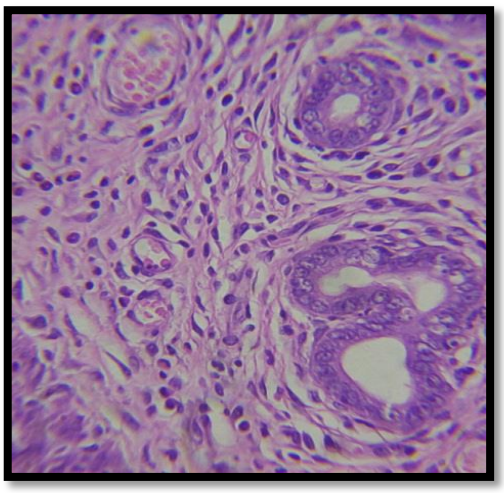


Plate b. Low dose group

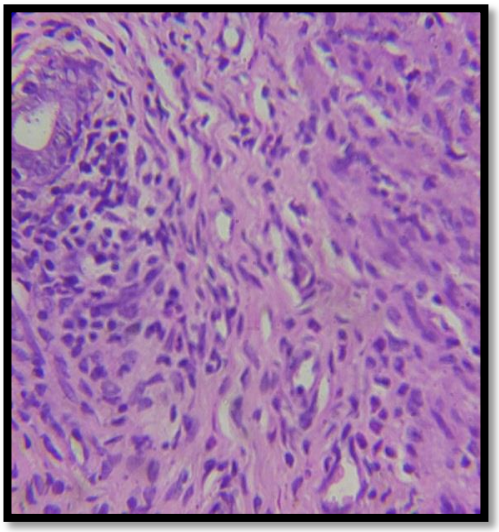


Plate c. Mid dose group

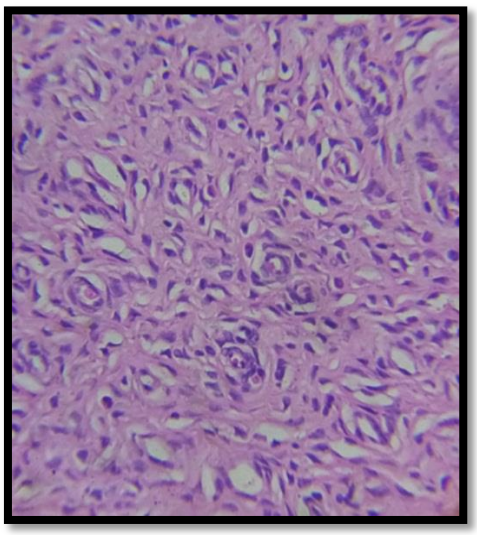


Plate d. High dose group

Plate e. Satellite group

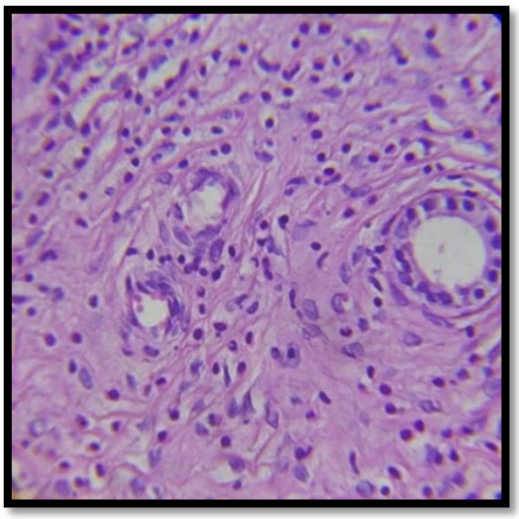


Plate a

- The appearance of endometrium, myometrium and uterine glands was normal.
- Endometrial gland, epithelium, and blood vessels appear normal.

Plate b

- The arrangement of uterine layers such Endometrium and myometrium are normal with no signs of abnormalities.
- Arrangement of stratum basal, functional and surface epithelium seems normal
- Endometrial gland, epithelium, and blood vessels appear normal.

Plate c

- The arrangement of uterine layers such Endometrium and myometrium are normal with no signs of abnormalities.
- Endometrial gland, epithelium, and blood vessels appear normal.

Plate d

- The arrangement of uterine layers such Endometrium and myometrium are normal with no signs of abnormalities.
- The arrangement of stratum basal, functional and surface epithelium seems normal.

Plate e

- The appearance of endometrium, myometrium and uterine glands was normal.

Sub-acute toxicity Study

Histopathology of Ovary

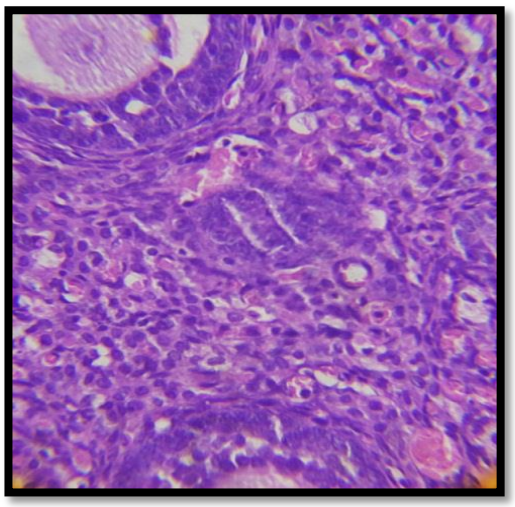


Plate a. Control

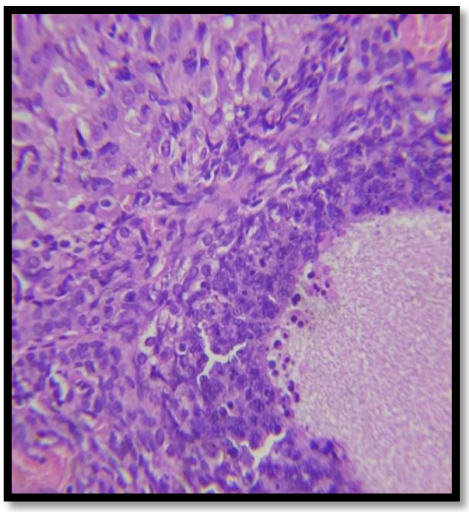


Plate b. Low dose group

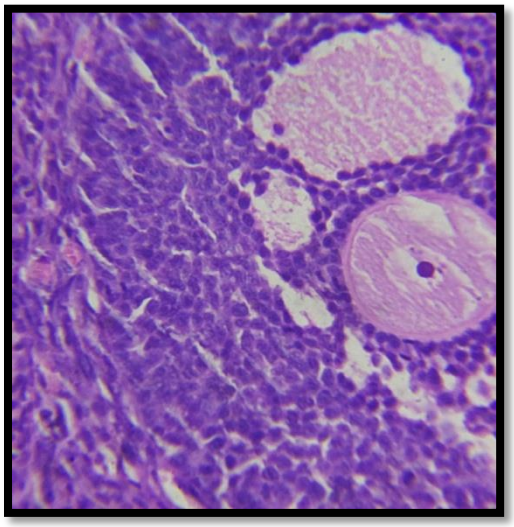


Plate c. Mid dose group

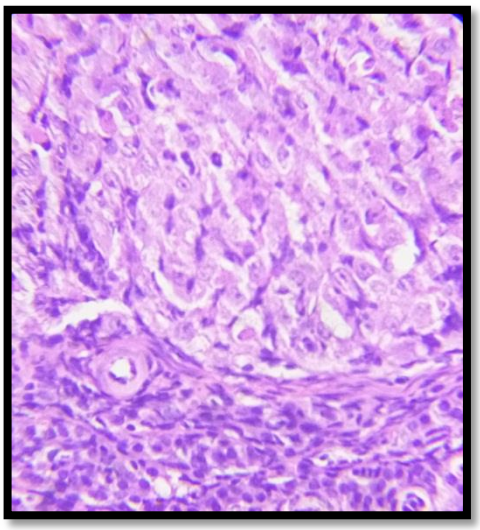


Plate d. High dose group

Plate e. Satellite dose group

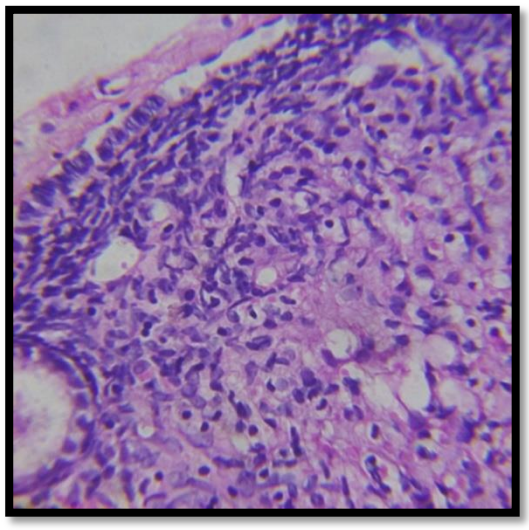


Plate a

- Histopathological analysis of ovary showing normal corpus luteum (CL) and Primordial follicles with few mature ovarian follicles with no signs of abnormality.

Plate b

- Sequential arrangement of granulosa cells around oocyte was normal and regular
- Follicular cells, cytoplasm, and nucleus appears normal
- Appearance of antral follicle, primary oocyte, and secondary follicles are normal

Plate c

- Histopathological analysis of ovary showing normal corpus luteum (CL) and Primordial follicles with few mature ovarian follicles with no signs of abnormality.

Plate d

- Sequential arrangement of granulosa cells around oocyte was normal and regular
- Follicular cells, cytoplasm, and nucleus appears normal

Plate e

- Sequential arrangement of granulosa cells around oocyte was normal and regular
- Follicular cells, cytoplasm, and nucleus appears normal

28 Day Repeated Dose Oral Toxicity Study

Histopathology of Brain

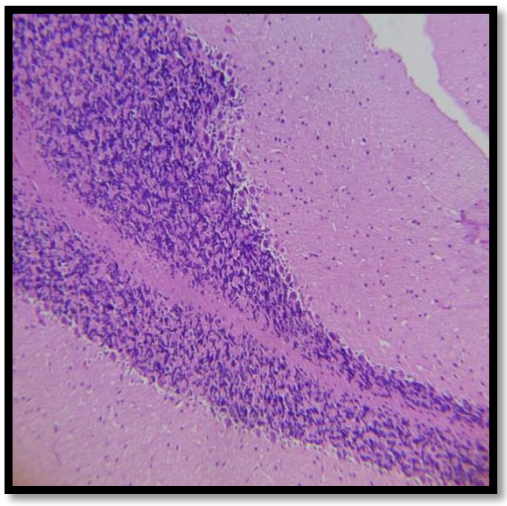


Plate a. Control

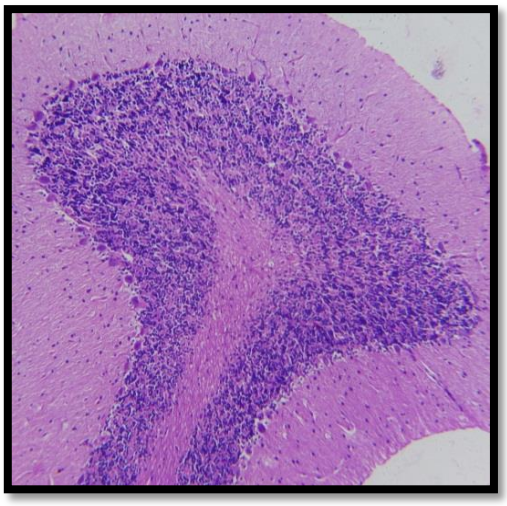


Plate b. Satellite group

Plate a

- Regular marginal alignment on the neurons with promising histology. Neuron is very intact and there were no signs of oedema or degeneration were observed.
- Section of cerebellum shows distinct molecular and granular layer. Neuronal architecture appears normal with sufficient numbers
- The arrangement of the neurons appears intact with no signs of degeneration or apoptotic changes.

Plate b

- Cortex region showed normal neurons with polygonal to round cell bodies containing dense cytoplasm.
- Normal appearance of cerebral cortex and medulla with intact parenchyma
- Neuronal cellular architecture appears normal with regular interneuronal space.

DISCUSSION

The **Tambira parpam** is used for the treatment of Earl kuruku veekam(hepatomegaly),Nadukuvatham (Parkinson's diseases),Maarpu selanthe (Breast tumour),Yonni putru (vaginal cancer),Gunmam(peptic ulcer),Pathavanmegam (filariasis) Naatpatta thalaivali (chronic headache)etc [9]under Siddha system of medicine. One of the ingredients of this drug was Tambiram, has a long history in the treatment of peptic ulcer and cancers among Siddha doctors in the Tamilnadu.

Literature Review reveals that there was no such research has been done on Thurusu sembu (Copper) parpam. As an initial step, in this present study, a part of standardization of this drug and its safety has been confirmed through necessary analysis and Acute & 28 days Repeated Oral Toxicity studies as per OECD guidelines.[7][8]

Standardization of the drugs means confirmation of its identity and determination of its quality and purity.[39]By way of standardization, metal-based drugs can be evaluated for their performances, limitations, optimal dosage, contraindications, and applications.[40] Stability parameters[39]such as Physical, Chemical for the herbo-mineral formulations were carried out for the test drug also.

Physico-chemical analysis (Table-1, 2) of Tambira parpam reveals that the drug appears Dark grey in colour and crystalline in nature. Its pH value was 8.97 Thus, this is a strong alkaline which is expected to have significant absorption in the in the stomach than in intestine. The loss on drying at $105^{O}C$ was 1.9160% w/w, which explains its moisture content leading to the decomposition of the plant materials. The Total Ash content and Acid Insoluble Ash were found to be 96.36% and 22.84% respectively. This explicates the purity of the test drug. Similarly, Water Soluble Extractive value and Alcohol Soluble Extractive value was found to be 5.56% and 0.81% respectively which makes clear that more amount of active constituents can be extracted with water as a solvent than alcohol is a solvent.

Qualitative analysis (Tables- 4,5,6) of Tambira parpam for Acid radicals, Basic radicals, and other constituents demonstrates the presence of Chloride, Phosphate, Iron, and Alkaloids.

The result of **Atomic Absorption Spectrometric Studies** (Table-7) of Tambira parpam for the determination of heavy metals proves that they are within the permissible limits as per WHO. The presence of Copper was found to be 11.2 ppm and others namely, Lead, Cadmium, Arsenic, and Mercury was below detectable limits.

Thermogravimetric analysis of Tambira Parpam carried out at the maximum of 1000^0C.The main objective of the study is to evaluate the decomposition and stability limit of the prepared formulation Tambira parpam. Prepared formulation Tambira parpam seems to be stable at the temperature varying from50 ºC to 800 ºC with no variation in the residual weight. The point of decomposition begins when the temperature increases beyond 800 ºC. Sharp deletion curve observed from 800 ºC to 825 ºC and at this point suggested crystal transformation may be observed. Predicted denaturation may be due to atomic change at oxygen atom present within the sample. The weight of the final residual matter was observed as 3.307 mg with 75.14% of residual volume. The remaining portion (approximately 25%) contains organic compounds also.

The **X-ray diffraction** pattern of the of the prepared formulation Tambira Parpam reveals the presence of a major peak with 2- Theta value of 38.719 which exactly matches to the ICDD (International Centre for Diffraction Data) 80- 1916. ICDD 80-1916 corresponds to the crystalline pattern of copper oxide (CuO). Hence the reference matching material was confirmed as copper oxide (CuO). Major peaks observed in Tambira parpam with 2-theta values of 35.49 and their corresponding intensities were 3492.The major peak observed in the reference matching material was 38.68 with the intensity value of 999. The XRD pattern of the test (Tambira Parpam) exactly matches with the reference material CuO, which justifies the presence of stable and purified CuO in the formulation. From the result of the present XRD analysis, it was concluded that the elemental composition of Tambira parpam confirms the presence of CuO at its stable state. Further copper being the major component of the formulation Tambira parpam.

Analysis of Tambira parpam by **Scanned Electron Microscopy** revealed the size stabilization of particles on the process and the presence of nano-sized particles with a range of 10.17nm to 22.9 µm. Nano- sized particles can attach to

the cell surface and can diffuse readily inside the cells. Thus, the size of the particle is able to influence the efficacy. The particles are Spherical in shape with a smooth surface. The particles show the evenly distribution in the fields examined (Figure – 1&2).

Analysis of Tambira parpam by **Fourier transform infrared spectroscopy (FTIR)** revealed the Infrared absorption pattern of CuO stretching was observed in the region of 523.16 cm−1 to 691.53 cm−1.Sharp absorption peak observed in the region of 590.87 cm−1 indicates the IR spectral pattern of CuO. Absorbance peak at 1107.93 cm−1 corresponds to CuO vibration due to the metal cation. The broad absorption peak at 3367.73 cm−1 corresponds to O-H stretching which is bonded. Wide absorption peaks at 1645 cm−1 may be due to the presence of primary amino group and also due to the vibrational intensity of C=C group. 1404 cm−1 corresponds to CH2 Bending. 1463 cm−1 corresponds to CH2 deformation.

In **Acute toxicity study** (Table - 9), carried out as per OECD guideline 423, there was no treatment- related death or signs of toxicity developed in albino rats at dosage levels of 5mg, 50mg, 300mg and 2000mg/kg body weight throughout the study period. Further, no gross pathological changes have been seen in the internal organs of both control and treated groups. Thus, the LD_{50} value was found to be greater than 2000 mg/kg body weight. With reference to the Globally Harmonised System of Classification and Labelling of chemicals, Tambira parpam can be classified as category-5[41] and this provides direct relevance for protecting human and animal health.

To ensure the safety of Tambira parpam, **28 days Repeated Oral Toxicity Study** was also carried out as per OECD test guideline 407. Except for hyperactivity at the time of drug administration, no other signs of toxicity were noted. After blood collection, all the animals were euthanized for gross pathological examinations of all major internal organs. The blood samples were sent to a lab for hematological and biochemical analysis. The organs were weighed and preserved in 10% buffered formalin solution before sending for histopathological study. All the reports were statistically analyzed.

Tambira parpam Significant difference in Food intake the test group animals were observed when compared with control group during the study period(Table8,9 & Figure3,4) but they are within physiological limit, and this study reveals that it does not adversely affect the basic metabolic processes of the experimental animals. The hemopoietic system serves as an important target for toxic chemicals and is a sensitive index for pathological conditions both in humans and animals. In Haematological parameters, it had been observed that WBC level was elevated after the administration of Tambira parpam at the high dose level (Table 12 & Figure 7, 8, 9, 10, 11, 12). But the WBC level was within normal range in post retrieval group after 14 days of withdrawal of medicine. Transaminases (SGOT and SGPT) are good indicators of liver function and biomarkers to predict the possible toxicity of drugs. Any elevation pertaining to these enzymes indicate their outflow into the blood stream due to damage in liver parenchymal cells. But, there was a marked increase in SGPT (Table – 15&Figure 15, 16) in high dose treated animals, when compared to control group, but it was also in normal range in Post retrieval group. In the present study, there was no treatment-related abnormality in renal functions at all the animals (Table - 14& Figure14) and other biochemical parameters (Table 13& Figure13) off Tambira parpam is treated animals were normal, when it is administered at higher dose level (400mg/kg). The therapeutic dose of Tambira parpam is 3mg/day for the human uses mentioned in Siddha text. This dose was very minimal compared to animal doses at low dose treated group (100 mg/kg b.wt)

The **histopathological study,** organs such as brain, heart, kidney, liver, lungs, spleen and stomach were taken. In organs of Control group, no abnormality was detected. In organs of Group III and IV, glomerular degeneration with mild derangement in mesenchymal density, alteration in thickness of proximal convoluted tubule mild tubular degeneration with increase bowman's space, renal tubule with the mild swollen epithelial cell were detected.

The extensive periportal degenerative change was observed. Hepatocytes rarely project dark pyknotic nuclei were observed in high dose groups. There's no pathological changes occur in other organs such as liver and renal, but post retrieval group of animals it not show any abnormalities during the study period.

SUMMARY

Tambira parpam is used for the treatment of Earl kuruku veekam (hepatomegaly), Nadukuvatham (Parkinson's diseases), Maarpu selanthe (Breast tumour), Yonni putru (vaginal cancer), Gunmam (peptic ulcer), Pathavanmegam (filariasis) Naatpatta thalaivali (chronic headache)&etc as mentioned in the Siddha literature. The raw drugs were procured from farms and shops. They were identified and authenticated by Botanist, National Institute of Siddha. The Raw drugs were purified and the medicine was prepared as mentioned in the Siddha literature. On organoleptic examination, the finished product seems to be grey in colour and crystalline in nature.

In physicochemical analysis of Tambira parpam, the pH was found to be in 8.97% with a loss on drying at 105^0C of 1.9160 % w/w.Total Ash value, Acid insoluble Ash value, Water, and Alcohol Soluble Extractive values reveal the purity of the test drug. **Qualitative Analysis** of Tambira parpam demonstrates the presence of Chloride, Phosphate, Iron, and Alkaloids.

Results of **AAS** confirm that the heavy metals were within the permissible limits in Tambira parpam, but copper content was present in 11.139 ppm level. **SEM** result confirms the presence of nano-sized, spherical shaped particles ranging between 10.17nm to 22.9µm, with a smooth surface in evenly distribution.

Thermogravimetric analysis of Tambira Parpam Weight of the final residual matter was observed as 3.307 mg with 75.14% of residual volume. The **X-ray diffraction** pattern of Tambira Parpam result of the present XRD analysis it was concluded that the elemental composition of Tambira parpam confirms the presence of CuO at its stable state. Further, copper is the major component of the herbo-mineral formulation Tambira parpam in oxide form.

Analysis of Tambira parpam by **Fourier transform infrared spectroscopy (FTIR**) revealed the Infrared absorption pattern of CuO stretching was observed in the region of 523.16 cm−1 to 691.53 cm−1 they a functional group of CuO, O-H group, Primary amino group and C=C group, CH_2 Bending and CH_2

deformation also observed in FTIR. This study also confirmed the presence of CuO in Tambira parpam.

The toxicological evaluations were conducted as per OECD guidelines 423 & 407 for safety evaluation of Tambira parpam . In acute toxicity study, no signs of toxicity and mortality were observed throughout the study period up to the dose of 2000mg/kg−body weight. Thus, the LD_{50} value of Tambira parpam was found to be greater than 2000 mg/kg body weight and classified as Category 5 [38]

In 28days Repeated dose Oral Toxicity Study, there was no significant changes in behavioral signs, food intake, water intake, Lipid Profile, Renal parameters hematological parameters (except WBC), and Hepatic parameters except that of SGOT and SGPT. The liver function test conducted at the end of the study, test groups (Low, Mid, High dose) revealed significant changes in level of liver parameters, when compared with control group animals. But the SGPT level of post retrieval animals were Normal limits compared to control group. There is no significant changes occurs in Satellite group. In Post retrieval group, both the Haematological and Hepatic parameters were observed in normal range.

In organs of Control group, no abnormality was detected. The histopathological changes were noted in liver and renal tissue at the dose level mid and High dose treated group. At the same time the normal histological structure present in post retrieval group of animals.

The above studies explained the qualitative of drug and presence of CuO in the study drug. They nanoparticles were also excises in the test drug by studding various analytical methods. From the safety study revealed that the high dose treated animals had some minimal pathological changes, but post retrieval animals had normal physiological parameters. So the drug may be eliminated after completion of the treatment period of the animals. These findings were crave the safety uses of the metallic preparation used in Siddha system of medicines.

CONCLUSION

Tambira parpam had been used by Siddhars for long time to treat various diseases such as Peptic ulcer, Breast tumour, Parkinson's diseases, hepatomegaly, vaginal cancer. Since Copper is present in Oxide form in Tambira parpam, which is observed by the quantitative analysis, the drug can be easily absorbed in intestine. Acute toxicity study shows that the test drug can be used up to the dose of 2000mg/kg bodyweight as a single dose. As per Siddha literature, the "test drug was used as a minimal dose medicine". Though administration of 200 mg/kg b.wt and 400 mg/kg b.wt Tambira parpam produces mild elevation of WBC and SGPT in Group Mid and High dose group of animals, no notable abnormalities were observed in Satellite group of animals. Hence, we conclude that the dosage of Tambira parpam, 1.5mg twice a day narrated in **Theran yemaha venba** [10] is a safer therapeutic dose for uses of human. The author hopes that this study will be a footprint to future research of Chronic toxicity study, Carcinogenicity, Teratogenicity regarding Tambira parpam.

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ANNEXURE

ANNEXURE

The following certificate are enclosed

- Research Methodology Certificate
- IAEC Certificate
- Authentication Certificate

BONAFIDE CERTIFICATE

Certified that I have gone through the dissertation submitted by **Dr. K. Anbarasan (Reg.No: 321416201)** a student of final year M.D(s), Branch-VI, Department of **Nanju Noolum Maruthuva Neethi Noolum**, **National Institute of Siddha**, Tambaram Sanatorium, Chennai - 47, and the dissertation work has been carried out by the individual only. This dissertation does not represent or reproduce the dissertation submitted and approved earlier.

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Name and Signature of the HOD (i/c),
Department of Nanju Noolum
Maruthuva Neethi Noolum,
National Institute of Siddha,
Tambaram Sanatorium,
Chennai - 47.

Name and Signature of the Director,
National Institute of Siddha,
Tambaram Sanatorium,
Chennai - 47.

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This is certify that the project title....Pre-clinical Safety.....
....evaluation of "TAMBIRA PARPAM" (15 Female Wister albino rats)
has **been approved by the IAEC.**(NO: NIS/IAEC-I/2016/09)

Name of ~~Chairman~~/Member Secretary IAEC:
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Name of CPCSEA

K. MACHIMUTHU

Signature with date

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Name of CPCSEA

K. MACHIMUTHU

Signature with date

24.2.2016

Chairman/Member Secretary of IAEC:

24. FEB 2016

CPCSEA nominee:

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

Tha Tamil Nadu Dr.M.G.R.Medical University

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This Certificate is awarded to Dr/~~Mr/Mrs~~.....K. Anbarasan.....

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25.05.2016

CERTIFICATE

Certified that the samples submitted for identification by Dr. K. Anbarasan, II year MD Student, Department of Nanju Nool, National Institute of Siddha, Chennai-600 047 is identified as Sembu – Copper.

(R. Shakila)
Research Officer (Chemistry)
~~I/c~~

(Dr. P. Sathiyarajeswaran)
Assistant Director (Scientist 2) I/c
सहायक निदेशक निदेशक Asst.Director I/c
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NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

Certified that the following plant drugs used in the Siddha formulation **"Tambira parpam"** (Internal) taken up for Post Graduation Dissertation studies by **Dr.K. Anbarasan**, M.D.(S), II year, Department of NanjuNoolum Maruthuva Neethi Noolum, 2016, are identified through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology, Micromorphology and Taxonomical methods as

Piper betle Linn. (Piperaceae), Leaf
Euphorbia nerifolia Linn. (Euphorbiaceae), Whole plant
Premna integrifolia Linn. (Verbenaceae), Leaves
Prosopis cineraria Linn. (Mimosaceae), Leaves
Calophylum inophylum Linn. (Clusiaceae), Seed
Gloriosa superba Linn. (Liliaceae), Tuber

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Date: 14-6-2016

Authorized Signatory

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